



# **Occurrence and Monitoring Document for the Final Ground Water Rule**



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## Acronyms and Notations

AFO	Animal Feeding Operation
AWWARF	American Water Works Association Research Foundation
AWWSCo	American Water Works Service Company
BGM	Buffalo Green Monkey
CAFO	Concentrated Animal Feeding Operations
CCL	Contaminant Candidate List
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CSO	Combined Sewer Overflow
CWS	Community Water System
CWSS	Community Water Systems Survey
DEP	Department of Environmental Protection
DNA	Deoxyribonucleic Acid
EA	Economic Analysis
EPA	U.S. Environmental Protection Agency
F+	Male-Specific
FBRR	Filter Backwash Recycling Rule (2001)
FRMK	Fetal Rhesus Monkey Kidney Cells
GWR	Ground Water Rule
GWUDI	Ground Water Under the Direct Influence of Surface Water
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
HPC	Heterotrophic Plate-Count Bacteria
ICC-PCR	Cell Culture-Polymerase Chain Reaction
IESWTR	Interim Enhanced Surface Water Treatment Rule (1998)
LT1ESWTR	Long Term 1 Enhanced Surface Water Treatment Rule (2002)
MAC	<i>Mycobacterium avium</i> Complex
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MPA	Microscopic Particulate Analysis
MPN	Most Probable Number
MPNIU	Most Probable Number of Infectious Units
MRDL	Maximum Residual Disinfectant Level
NCWS	Non-Community Water System
NPDWR	National Primary Drinking Water Regulations
NTNCWS	Non-Transient Non-Community Water System
OPR	Coliphage-Positive Reagent Water
OST	Office of Science and Technology
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Units
PSE	Pfizer Selective Enterococcus
PWS	Public Water System
QA/QC	Quality Assurance and Quality Control
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAL	Single Agar Layer
SDWA	Safe Drinking Water Act

SRSV	Small Round Structured Viruses
Stage 1 DBPR	Stage 1 Disinfectants and Disinfection Byproducts Rule (1998)
SWTR	Surface Water Treatment Rule
TC	Total Coliforms
TCR	Total Coliform Rule (1989)
TNCWS	Transient Non-Community Water System
TSB	Tryptic Soy Broth
UIC	Underground Injection Control
UMCR	Unregulated Contaminant Monitoring Rule
USGS	United States Geological Survey
VBNC	Viable But Non-Culturable

## **1.0 Introduction**

### **1.1 Background**

The Safe Drinking Water Act (SDWA) ensures protection of the nation's public drinking water supply. Under the SDWA, the United States Environmental Protection Agency (EPA or Agency) is promulgating a drinking water regulation, the Ground Water Rule (GWR), to refine control of microbial pathogens in public water systems (PWSs). The primary goal of the GWR is to identify ground water PWSs that are susceptible to fecal contamination, particularly at their sources, and to ensure that they take adequate measures to remove or inactivate pathogens in drinking water they provide to the public.

The control of microbial contaminants in drinking water supplies using ground water is complicated. This is because there are a very large number of PWSs using ground water, many of which are very small. In addition, there are a substantial number of microbial contaminants of concern and pathways of contamination, and no single approach for controlling pathogens is universally applicable. Thus, EPA developed several major rule components under the GWR:

- Periodic sanitary surveys of ground water systems requiring the evaluation of eight elements and the identification of significant deficiencies
- Source water monitoring (5 source water samples) for wells with a positive fecal indicator sample, with corrective action required upon a second fecal indicator-positive sample
- Optional hydrogeologic assessments to identify wells sensitive to fecal contamination
- Optional source water monitoring for all systems, including systems drawing from sensitive wells without treatment or with other indications of risk
- Compliance monitoring to ensure disinfection treatment (where used) is reliably operated
- Correction of significant deficiencies and fecal contamination through one or more of the following actions:
  - Eliminate the source of contamination
  - Correct the significant deficiency
  - Provide an alternative source of water
  - Provide a treatment which achieves at least 99.99 percent (4-log) inactivation or removal of viruses

Alternative public health protection strategies, such as closure of pathogen-contaminated wells, are not precluded by the GWR.

### **1.2 Ground Water Rule Statutory Authority**

The SDWA establishes mandates for protecting the nation's public drinking water supply. The 1986 amendments to the SDWA directed EPA to establish National Primary Drinking Water Regulations (NPDWRs) requiring disinfection as treatment for the inactivation of microbiological contaminants for all PWSs, including systems supplied by ground water sources. This mandate was amended in the 1996

amendments to SDWA (Section 1412(b)(8)) to require disinfection for ground water sources “as necessary.”

EPA has the responsibility to develop a ground water rule which not only specifies the appropriate use of disinfection but, just as importantly, addresses other components of ground water systems to ensure protection of public health. Section 1412(b)(1)(A) of the SDWA requires EPA to establish NPDWRs for contaminants that “may have an adverse effect on the health of persons,” are “known to occur or there is a substantial likelihood that the contaminant will occur in PWSs with a frequency and at levels of public health concern,” and for which, “in the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reductions for persons served by PWSs.” In response to the 1996 SDWA Amendments, EPA proposed (65 FR 30194; May 10, 2000) and is now finalizing the GWR to address the problem of microbial pathogen contamination of ground water-supplied drinking water systems.

### **1.3 Purpose of the Occurrence and Monitoring Document**

This document, the *Ground Water Rule Microbial Occurrence and Monitoring Document* is one of six regulatory support documents issued in conjunction with promulgation of the GWR. The other five documents are the:

- Technology and Cost Document
- Economic Analysis
- Information Collection Request
- Response to Public Comments Document
- Ground Water Rule Peer Review Report

In addition to the support documents, EPA intends to develop six guidance documents to assist systems and states in implementation of the GWR:

- Hydrogeologic Sensitivity Assessment Guidance
- Sanitary Survey Guidance
- Corrective Action Guidance
- Small Systems Implementation Guidance
- Source Water Monitoring Guidance
- Individual and Non-Public Water Supply Systems Guidance

Information from the occurrence and monitoring document provides background for all GWR regulatory support and guidance documents, but is used primarily to support analyses of the national risks and the national costs and benefits presented in the Economic Analysis (EA). This document provides

background information regarding the contaminants in drinking water sources regulated under the GWR, including descriptions of the following:

- The nature and occurrence of viral and bacterial pathogens
- Various potential microorganism indicators of fecal contamination
- Health risks associated with exposure to viral and bacterial pathogens
- The links between pathogenic and indicator microorganisms
- How microbial contamination of ground water systems occurs
- Microorganism fate and transport
- Standard methods for monitoring microorganisms that are used to study microbial occurrence and to identify microorganisms as part of rule requirements

Viral and bacterial pathogens and indicator occurrence data are important to the GWR for several reasons. First, pathogen occurrence in an untreated PWS well represents an unequivocal indicator that there is a public health risk, and immediate action, such as well closure or disinfection, is necessary. Second, national occurrence estimates of pathogens in wells, when combined with dose-response data for those pathogens, yields a national estimate of public health risk. Third, differential assessment of potential public health risk can be evaluated using pathogen and/or indicator occurrence data, thereby meeting the SDWA mandate to disinfect “as necessary.” Finally, indicator occurrence data can be used, in an appropriate monitoring framework, to identify wells where corrective action may be needed.

## **1.4 Ground Water, Surface Water, and Distribution System Regulatory Requirements**

The GWR is one of several rules designed to protect PWSs from microbial contamination of surface water sources, ground water sources, and drinking water distribution systems. Plants using surface water or ground water under the direct influence of surface water (GWUDI) are regulated under the Surface Water Treatment Rule (SWTR), the Interim Enhanced Surface Water Treatment Rule (IESWTR), the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR), the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), and/or the Filter Backwash and Recycling Rule (FBRR). These rules were designed to protect against contamination by the waterborne protozoans *Giardia* and *Cryptosporidium*, bacteria, and viruses. Although these protozoans have occurred in ground water PWSs (Hancock et al. 1998) and have caused outbreaks in such systems (Solo-Gabriele and Neumeister 1996), they are not addressed under the GWR because EPA considers wells where these organisms are present to be GWUDI and regulates these sources under the rules above.

Other regulations exist that apply to PWSs drawing water from both ground water and surface water sources, such as the Total Coliform Rule (TCR) and the Stage 1 Disinfectants and Disinfection Byproducts Rule (DBPR). The TCR requires distribution system monitoring for total coliforms (TCs) and the Stage 1 DBPR sets a maximum residual disinfectant level (MRDL) for disinfecting PWSs. Like the GWR, these rules address issues of microbial contamination of drinking water. However, the GWR plays an important role in addressing issues that may not be adequately covered under these rules. Specifically, the GWR addresses potential contamination in ground water systems not achieving 4-log treatment of viruses and addresses the issue at or before the first customer.

Although not the basis for the GWR, there are additional waterborne pathogens that EPA has evaluated as part of the Contaminant Candidate List (CCL). These pathogens are typically bacteria that are either free-living in the environment or bacteria that can colonize pipes of drinking water distribution systems [(e.g., *Legionella* (causes Legionnaires Disease, Pontiac Fever), *Pseudomonas aeruginosa*, *Mycobacterium avium-intracellulare*)]. Future refinements to the CCL may include other waterborne pathogens. EPA recognizes the potential risks from such organisms, but believes that more research needs to be conducted before they can be properly considered for regulation. The GWR is not specifically targeting these organisms or the water distribution system ecosystems that they tend to colonize. However, it is likely that the protections offered by the GWR may also provide some benefits against bacterial contamination in distribution systems. For example, sanitary surveys may identify nutrient sources that nourish colonizing bacteria and may identify methods to mitigate nutrient influx.

## 1.5 Document Organization

This document is organized into seven chapters and an Appendix. A description of each remaining chapter and the appendix is presented below.

- **Chapter 2—Public Health Hazard:** Microbial contaminants, indicator bacteria, and viruses of interest are briefly discussed. Waterborne disease outbreak data and TCR violations are presented.
- **Chapter 3—Sources of Fecal Contamination of Ground Water and Wells:** The nature of microbial contamination sources are discussed. Outbreak information is presented for those outbreaks for which microbial pathogen contamination sources have been identified, along with any available data on occurrence of outbreak-causing organisms in water samples collected during an outbreak.
- **Chapter 4—Microbial Contaminant Fate and Transport:** The capability of microbial contaminants to survive in the environment is discussed and virus and bacteria subsurface travel distances are presented. Waterborne disease outbreak information is presented with a focus on hydrogeologic settings that facilitate microbial pathogen contaminant survival and transport to an untreated (or inadequately treated) drinking water well, thereby causing an outbreak.
- **Chapter 5—Microbial Contaminant Monitoring:** Monitoring methods for microbial pathogens and indicator organisms are discussed.
- **Chapter 6—Occurrence Analysis:** Important or relevant microbial occurrence data are presented based on a recent scientific literature review. Studies that best describe and represent national occurrence were evaluated and are presented along with resulting national occurrence estimates.
- **References:** Supporting scientific literature is identified.
- **Appendix A:** Includes descriptions of new monitoring methods being developed.

## 2.0 Public Health Hazard

### 2.1 Introduction

The Ground Water Rule (GWR) applies to all public water systems (PWSs) served solely by ground water, except for PWSs that are designated as ground water under the direct influence of surface water (GWUDI), since such systems are subject to the regulations governing surface water sources. The rule also applies to PWSs that mix surface and ground water if the ground water is added directly to the distribution system and provided to consumers without the treatment received by the surface water source. EPA also recommends that private wells that serve large populations (fewer than 60 days per year) conduct frequent monitoring. This document addresses the public health hazards for PWSs using ground water sources.

In the application of National Primary Drinking Water Regulations (NPDWRs) to PWSs, the regulations usually make a distinction between community and non-community systems. A further distinction is made between transient and non-transient non-community systems. This document uses definitions in evaluating occurrence and exposure as follows:

- A **PWS** is defined as a system that provides water for human consumption through pipes or other conveyances if such a system has at least 15 service connections or regularly serves an average of at least 25 individuals per day for at least 60 days per year.
- **Community water systems** (CWSs) serve a residential population of at least 25 people or 15 service connections on a year-round basis. Users of community systems are likely to be exposed to any contaminants in the water supply over an extended time period, and are thus subject to both acute and chronic health effects.
- **Non-community water systems** (NCWSs) do not serve permanent residential populations. Non-community systems are either transient or non-transient. **Non-transient non-community water systems** (NTNCWSs) serve at least 25 of the same persons at least 6 months per year on a regular basis. These systems can expose users to drinking water contaminants over an extended time period (subjecting users to risks of both acute and chronic health effects), similar to community systems. Schools and factories having their own water supplies would typically fall under this definition. **Transient non-community water systems** (TNCWSs) serve short-term users. As a result, the users are exposed to any drinking water contaminants only briefly. Users are subject to experiencing mainly acute health effects. Examples of TNCWSs include restaurants, gas stations, hotels, and campgrounds (provided that the workers at these establishments are too few to meet the definition of a non-transient system).

The GWR does not apply to private wells (wells that do not meet the definition of a PWS). However, EPA recommends private well owners test for coliform bacteria once each year.

The purpose of this section is to briefly describe the nature of the public health hazards<sup>1</sup> for drinking water consumers created by consuming or being otherwise exposed to ground water provided by

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<sup>1</sup> A quantitative assessment of the public health risk is available in the *Economic Analysis for the Final Ground Water Rule* (USEPA 2006a).

PWSs. For GWR evaluation, EPA estimated exposure to ground water sources by compiling data from a number of sources, including the Safe Drinking Water Information System (EPA 2003) and the 1995 Community Water System Survey (CWSS) (USEPA 1997). Data indicate that there are 147,330 PWSs served by primarily ground water (42,361 CWSs, 18,908 NTNCWSs, and 86,061 TNCWSs) in the United States, serving approximately 115 million people (100 million served by CWSs, 5 million served by NTNCWSs, and 9 million served by TNCWSs).

Traditionally, ground water that has not been recently recharged by surface water has been considered safe for drinking without treatment to remove microbial pathogens because of the natural filtration provided by soil and aquifer materials. Where used to good effect, these zones provide protection by providing in-situ treatment that reduces pathogen concentrations. Therefore, many PWSs served by ground water do not now disinfect or otherwise treat their drinking water. However, the capacity of soil and aquifer materials to attenuate pathogens depends on the aquifer properties, the pathogen concentration in the recharge water, and the location of the contamination source. For example, in areas where septic tank drain fields and other human or animal waste management operations perform poorly, pathogen concentration from one or multiple sources is high, and if ground water flow is rapid and direct through the aquifer, there is greater possibility of a public health hazard.

If pathogenic viruses or bacteria remain viable in ground water and are ingested in sufficient numbers at the point where drinking water is delivered to the customer, illness can occur. Many of these pathogens are the result of fecal contamination of ground water, described below. However, a few waterborne pathogens, such as *Legionella*, may also occur naturally in ground water or enter the water system through other means. These pathogens cause illness through ingestion, inhalation, person-to-person contact or dermal (skin) exposure (from air or contaminated water or surfaces). For example, inhalation of *Legionella* bacteria causes Legionnaire's disease, a form of pneumonia, and some cases of conjunctivitis, an eye infection, are caused by bacterial or viral contamination of wash water.

Fecal contamination is a very general term that includes all of the organisms found in feces (both pathogenic and non-pathogenic). It also includes organisms found in sewage, septage, agricultural wastes, wastewater, and septic drainage. Microorganisms found in fecal matter are also called enteric microorganisms because they live in the human gastrointestinal system.

Fecal contamination of ground water can occur by several routes. First, fecal contamination can reach the ground water source from failed septic systems, leaking sewer lines, and from passage of fecal matter through soils, soil fissures, or directly into bedrock openings where soils are absent. The volume of septic tank waste that is released into the U.S. subsurface has been estimated at 1 trillion gallons per year (Canter and Knox 1984). This discharge may eventually reach the subsurface intake zone of a drinking water well. Second, fecal contamination from the surface may enter the well along the casing or through cracks in the sanitary seal if it is not properly constructed, protected, or maintained. Third, fecal contamination may also enter the distribution system when cross-connection controls fail or when negative pressure in a leaking pipe allows contaminant infiltration.

Distribution systems themselves may harbor bacterial pathogens (e.g., in biofilms, bacterial microcolonies that form slimes inside distribution systems), especially the opportunistic pathogens that cause illness primarily in individuals with weakened immune systems. These bacterial pathogens may have entered the distribution system via fecal matter through storm water infiltration, cross connection with sewer lines, or other undetermined methods. The source of fecal matter may be humans or other animals. Biofilms may also harbor viral pathogens (Quignon et al. 1997). Viruses do not reproduce in the biofilm, although they may be protected by the film against disinfectants and, thus, survive longer than they would in a normal distribution system environment.



Many bacterial pathogens may infect both humans and animals; thus, animal fecal material may contain bacterial pathogens that affect humans. In contrast, enteric viruses that have human hosts primarily infect only humans. Opportunistic pathogens, like those harbored in biofilms, cause illness in sensitive subpopulations, such as the elderly or immunocompromised. All pathogens may, in unusual cases or in sensitive subpopulations, infect a variety of sites within the human body, rather than only the intestinal tract.

The following sections describe the microbial contaminants that could be found in ground water PWSs, the occurrence of disease outbreaks associated with ground water systems, and contamination routes to those drinking water sources.

## **2.2 Microbial Contaminants in Ground Water**

This section describes the microbial contaminants (either pathogens or indicator organisms) for which occurrence data or other pertinent information pertaining to the public health risk are available for PWSs using ground water in the United States.

### **2.2.1 Waterborne Pathogenic Bacteria, Viruses, and Other Pathogens**

Waterborne pathogens commonly travel the fecal-oral route of transmission, where the microorganisms replicate in a host, the host sheds the pathogens in fecal material that enters a water body used for drinking water, and a person ingests the water and organisms and becomes infected. Pathogens may also enter the water through other means and infect humans through other routes.

Over 120 types of harmful enteric viruses are excreted in human feces. Enteric viral pathogens are excreted almost exclusively by humans (Gerba 1988), usually for short durations, which may last for a few days to a few weeks (Grabow 2001). Viral pathogens may be pathogenic even when very few viral particles are ingested. The human enteric virus concentrations in environmental waters depend on a number of variables, including the number of epidemic or endemic infections, vaccination against viruses, and seasonal effects (Grabow 2001). However, pathogenic viruses do not survive long (greater than about one year), or replicate in environmental waters because they need living cells to replicate and grow. Bacterial pathogens of fecal origin probably do not survive as long as viruses in ground waters but are perhaps more likely to be able to replicate, albeit perhaps not in significant numbers (Pedley et al. 2006).

Enteric waterborne pathogenic bacteria and viruses identified by medical laboratories working with fecal samples from U.S. drinking water consumers are listed in Exhibit 2.1. Waterborne pathogenic enteric viruses identified include polioviruses, coxsackieviruses, enteroviruses, adenoviruses, rotavirus, hepatitis A virus, hepatitis E virus, noroviruses, astroviruses, and sapoviruses. Certain species of *Shigella*, *Vibrio*, *Salmonella*, *Yersinia*, *Campylobacter*, *E. coli*, and *Arcobacter* bacteria have been detected in fecal samples. *Legionella*, *Mycobacterium*, and *Helicobacter* have also been identified in sputum or other types of samples. Each of these organisms are shed in relatively large numbers from infected individuals or may be harbored in distribution systems, and they are capable of infecting humans. Each is capable of being transmitted via water, although, for a few viruses, (e.g., astrovirus, hepatitis A virus, and enterovirus 70 and 71), water may not be the primary route of transmission.

## Exhibit 2.1 Pathogenic Microbial Contaminants

Waterborne Pathogenic Bacteria	Waterborne Pathogenic Viruses
<i>Legionella</i> <i>Mycobacterium avium</i> Complex (MAC) <i>Shigella</i> (several strains) <i>Helicobacter pylori</i> <i>Vibrio cholerae</i> <i>Salmonella typhi</i> <i>Salmonella typhimurium</i> <i>Yersinia</i> <i>Campylobacter</i> (several strains) <i>Escherichia coli</i> ( <i>E. coli</i> ) (several strains) <i>E. coli</i> O157:H7 <i>Arcobacter butzleri</i>	Enteroviruses Coxsackieviruses Echoviruses Poliovirus Enterovirus 70 & 71 and other enteroviruses Hepatitis A virus (HAV) Hepatitis E virus Adenovirus Rotavirus Norovirus Astrovirus Sapovirus

Source: Adapted from Grabow 2000; USEPA 2001a; Lederberg 1992; Craun 1996; and Craun and Calderon 1996.

Three pathogenic viruses in Exhibit 2.1 (echoviruses, coxsackieviruses, and caliciviruses) and *Helicobacter pylori*, a bacterial pathogen, were on the January 11, 2001, Unregulated Contaminant Monitoring Rule (UCMR) list. The list identified contaminants that were being considered for the Candidate Contaminant List (CCL) for drinking water; these contaminants were later added to the CCL. The CCL is a list of contaminants that are not subject to NPDWRs, but may require future regulation. Monitoring requirements for any of the pathogens, if regulated, will be established in future rulemakings after adequate test methods have been developed.

For some pathogens, it is sometimes difficult to obtain data confirming the link between a ground water source and the specific agent attributed to the outbreak. For instance, no routine, commercially available test for identifying norovirus exists. Hopkins et al. (1985, 1984) confirmed the link between rotavirus and ground waterborne disease in outbreaks that occurred in Colorado. Kukula et al. (1997) first reported a drinking water outbreak of adenovirus affecting up to 3,000 people in a Finnish municipality due to contaminated ground water. Subsequently, CDC (2005a) identified adenovirus in ground water samples during the South Bass Island, Ohio, outbreak (Ohio EPA 2005).

One reason it is difficult to establish links between water sources and outbreaks is that outbreaks are often underreported because those infected do not always seek medical care. However, a recent study of patients visiting the emergency room for diarrhea in New Haven, Connecticut, and Baltimore, Maryland, determined that norovirus accounted for 11.8 percent and rotavirus 10 percent of patients with diarrhea (Nataro et al. 2006). One *E. coli* strain, the enteroaggregative strain, was responsible for 4.5 percent of cases. Other bacteria present in case patients included *Campylobacter jejuni* at 2.9 percent, *Salmonella* at 2.0 percent, and *Clostridium difficile* at 1.9 percent.

To protect public health, EPA sets a maximum contaminant level (MCL) for disease-causing drinking water constituents. The maximum contaminant level goal (MCLG), a non-enforceable public health goal, is the level of contaminant in drinking water below which there is no known or expected risk to health. The MCLG for viruses is zero. Because of the inherent difficulties described above for enumerating pathogenic viruses, the GWR MCL for enteric viruses is a “treatment technique” standard (e.g., chlorination for systems, as needed) that requires 4-log inactivation of viruses in ground water used for drinking water. To complement the treatment technique, the GWR’s protection against illnesses also

includes monitoring for fecal contamination indicators, as well as sanitary surveys to identify potential threats to drinking water systems. Fecal contamination indicators are discussed in the next section.

### **2.2.1.1 Pathogenic Bacteria**

#### ***E. coli***

The group of bacteria known as *E. coli* contain both pathogenic and non-pathogenic isolates. Non-pathogenic *E. coli*, also called commensal *E. coli*, occur naturally in the human intestine. Pathogenic *E. coli* may cause kidney failure, death, and chronic kidney problems, as described further in this paragraph, as well as cramps and diarrhea (often bloody). The most dangerous *E. coli* bacteria contain the gene for producing shiga toxins. *E. coli* O157:H7 is the most widespread shiga-toxin producing *E. coli*, but at least 81 serotypes have been identified (Prager et al. 2005). Release of toxins in the body can result in kidney failure, shock, and death in otherwise healthy individuals, especially small children. Typically, kidney failure occurs in 2-7 percent of illnesses. Death or end-stage renal disease occurs in about 12 percent of patients four years after diarrhea-associated kidney failure (Garg et al. 2003). Twenty-five percent of kidney failure survivors demonstrate long-term renal problems, or sequelae (Garg et al. 2003). For patients with moderate and severe gastroenteritis caused by *E. coli*, long-term study shows that they have an increased risk of hypertension and reduced kidney function (Garg et al. 2005). CDC estimates that drinking water is responsible for 15 percent of the 73,000 illnesses each year from *E. coli* O157:H7 in the United States (Rangel 2005). *E. coli* O157:H7 has been implicated in three waterborne disease outbreaks in ground water PWSs (one system that uses wells and two that use springs) (Swerdlow 1992; Levy et al. 1998). Another serotype, *E. coli* O86a:H11, is significantly associated with a waterborne disease outbreak at a PWS using ground water (NM State Department of Health 1998).

Although manure is often considered to be the source of shiga-toxin producing *E. coli*, they have also been isolated from municipal sewage (Holler et al. 1999). *E. coli* O157:H7 was found to survive on a pasture surface for almost 4 months. About 4 to 15 percent of cases are acquired via secondary transmission (Parry and Salmon 1998). In addition to the shiga-toxin producing *E. coli*, a number of pathogenic *E. coli* bacteria produce other toxins. Hunter (2003) identifies 62 *E. coli* strains capable of causing diarrheal disease. Little data are available on the hazard associated with waterborne transmission for most of the pathogenic *E. coli* other than *E. coli* O157:H7.

Although *E. coli* is monitored in PWSs via the Total Coliform Rule, the methods currently approved by EPA for the rule are not capable of identifying shiga toxin-producing *E. coli*. Because *E. coli* are often found in ground water supplies, and because low doses can result in infection, it is likely that disease due to *E. coli* is quite prevalent in association with PWS wells; however, few data on pathogenic *E. coli* in PWS wells are available.

#### ***Shigella***

*Shigella* is one of the most commonly recognized causes of waterborne disease outbreaks because the advent of bloody diarrhea spurs detailed investigations and a cause is often identified, although *Shigella* is difficult to cultivate. *Shigella* also produces shiga toxins; in fact, the shiga toxin-producing *E. coli* bacteria acquired the capability to produce toxins by exchanging plasmids (DNA segments) with *Shigella*. Thus, as does shiga toxin-producing *E. coli*, *Shigella* often also causes kidney failure and chronic kidney disease. *Shigella* contamination is probably less widespread than *E. coli* contamination because only humans carry *Shigella*. One large ground water outbreak in a sensitive aquifer occurred recently in Island Park, Idaho, possibly caused by an unidentified broken sewer line contaminating the well water (CDC 1996).

## ***Campylobacter and Arcobacter***

*Campylobacter* is a very common contaminant of food and water. *Campylobacter* is commonly associated with animal manure, especially cow and chicken manure. Uniquely, *Campylobacter* is often associated with Guillain-Barre paralysis, which can last for weeks or months. About 1 paralysis case occurs for every 1000 cases of campylobacteriosis (Altekruse et al. 1999). About 20 percent of paralysis patients are left with some disability and approximately 5 percent die. Campylobacteriosis is also associated with Reiter syndrome (reactive arthritis). Approximately 1 percent of patients with campylobacteriosis have arthritis onset in one or more joints (especially the knee) in the 7 to 10 days after diarrheal onset (Altekruse et al. 1999).

*Campylobacter* was associated with the recent outbreak in South Bass Island, Ohio, caused by widespread fecal contamination in a sensitive aquifer (Ohio EPA 2005). The *E. coli* O157:H7 outbreak in Walkerton, Ontario, was also a large outbreak of campylobacteriosis. *Arcobacter* (now a separate genus from *Campylobacter*) was responsible for a ground water outbreak at a camp in Coeur d'Alene, Idaho (McMillan 1996), where the sensitive aquifer was contaminated by a septic tank.

As with all bacterial pathogens, special enrichment methods are needed to identify *Campylobacter* and *Arcobacter* in environmental samples, so no data are available on their general occurrence in PWS well water. *Campylobacter* has a viable but non-culturable environmental form, which makes it difficult to detect at times in water (Rollins and Colwell 1986, Koenraad et al. 1997).

## ***Salmonella***

*Salmonella typhi* causes typhoid fever, once a common and dangerous waterborne disease, and *Salmonella typhimurium* causes salmonellosis. Typhoid is now rare in the United States due to improved sanitation and water treatment, but salmonellosis still exists. In recent years, *Salmonella* has become increasingly less common as a common source outbreak agent while campylobacteriosis outbreaks have correspondingly increased. The reasons for this change are unclear. *Salmonella* DNA was identified in most fecally contaminated PWS wells during the South Bass Island, Ohio, outbreak in 2004 (Ohio EPA 2005). However, many drinking water *Salmonella* outbreaks result from scenarios other than source water contamination. For example, the seven deaths that occurred due to *Salmonella* contamination in a ground water system in Gideon, Missouri, were due to bird entry into a storage tank (Angulo et al. 1997). *Salmonella* resulted in a very large outbreak in a ground water utility in Riverside, California during the 1960s (16,000 illnesses, 70 hospitalizations and 3 deaths) prior to the advent of the Total Coliform Rule (Boring et al. 1971).

## ***Legionella***

*Legionella* are opportunistic bacterial pathogens that colonize water distribution systems. Infection usually occurs through inhalation of mist, particularly from cooling towers used for industrial cooling. An estimated 8,000-10,000 cases of Legionnaire's disease (a type of pneumonia) and Pontiac fever (characterized by fever and muscle aches) occur in the United States each year due to *Legionella*. Twenty-one of 48 known species are able to infect humans. A study of 46 PWS wells from 16 water utilities in the United States and Canada showed that 38 wells (82 percent) were positive for *Legionella*. About half the identified *Legionella* species were pathogenic. The authors conclude that this is the "first study that has unambiguously proven that *Legionella* constitute a part of the microflora of ground water not known to be under the direct influence of surface waters" (Riffard et al. 2004).

Some people who contract acute endemic gastrointestinal illness are seriously affected by other diseases caused by the same organism, and some die. Danish researchers who had access to national

health and civic registries determined a national mortality rate for patients who had had foodborne bacterial gastrointestinal illnesses (Helms et al. 2003). Of 48,847 patients, 55.2 percent had non-typhoidal *Salmonella*, 33.1 percent had *Campylobacter*, 8.3 percent had *Yersinia enterocolitica*, and 3.4 percent had *Shigella*. (*E. coli* was not mentioned in the article.) One year after infection, the death rate was 3.1 times higher for cases than for controls. Complications were often due to invasive illnesses occurring within 30 days of infection, including septicemia, endocarditis, pneumonia, and meningitis.

### **2.2.1.2 Pathogenic Viruses**

#### ***Enterovirus***

Enteroviruses include polioviruses, coxsackieviruses, echoviruses, and others. Many are transmitted via the fecal-oral route, although they may also be transmitted through contact with respiratory secretions and saliva (CDC 2005b). According to the Centers for Disease Control and Prevention (CDC), enteroviruses are more common than any other virus except rhinovirus (the common cold virus) (CDC 2005b). Most enteroviruses cause mild upper respiratory symptoms or flu-like symptoms, although infected people may experience no symptoms at all. Enteroviruses can also cause viral meningitis, characterized by inflammation of the tissue surrounding the brain and spinal cord. Viral meningitis may require hospitalization and myocarditis. Enteroviruses can also cause encephalitis (inflammation of the brain) and myocarditis (infection of the heart). In addition, mothers can pass enterovirus infections to their babies during childbirth; this infection is sometimes fatal (CDC 2005b). Chronic health effects associated with enteroviruses are described in section 2.2.1.4.

#### ***Rotavirus***

Rotaviruses are another very common virus, responsible for 55,000-70,000 hospitalizations annually among children in the United States. Rotavirus causes severe diarrhea, especially in infants and very young children, that lasts for 3–7 days; symptoms may also include vomiting and fever. An estimated 20-60 deaths occur per year in the United States among children less than 5 years old (CDC 2006). Infection in adults is usually not as severe. Transmission occurs mostly via the fecal-oral route. In 2006 the FDA approved a rotavirus vaccine (CDC 2006).

#### ***Norovirus***

Norovirus, a subset of calicivirus, is recognized as an agent that imposes a substantial disease burden on both the United States and other developed countries (Mead et al. 1999, Carter 2005, Turcios et al. 2006). According to Carter, analysis suggests that noroviruses are “the single most significant cause of intestinal infectious disease in the developed world.”

The health effects of norovirus illness include acute onset of nausea, vomiting, abdominal cramps, and diarrhea. Vomiting is more prevalent among children, whereas a greater proportion of adults also experience diarrhea. Headache, fever, chills, and myalgia are frequently reported. Although rare, severe dehydration caused by norovirus gastroenteritis can be fatal, with this outcome occurring among susceptible persons (e.g., older persons with debilitating health conditions). No long-term sequelae of norovirus infection have been reported (CDC 2001a). Duration of illness is typically 12–60 hours.

In recent years, numerous common source outbreaks<sup>2</sup> have been attributed to norovirus contamination. CDC retrospectively evaluated 4,050 U.S. common source outbreaks that occurred during 1998–2000 and determined that, at a minimum, 28 percent could be attributable to norovirus (Turcios et al. 2006). In another study of outbreaks occurring from 2000 to 2004, fecal samples from 226 outbreaks (12 waterborne) of acute gastroenteritis suspected of calicivirus causality were analyzed by CDC for norovirus and other caliciviruses (Blanton et al. 2006). Caliciviruses (primarily norovirus but also sapovirus) were detected in 184 (81 percent) of those outbreaks. These data suggest that norovirus represents a large component of the total gastroenteritis epidemic disease burden in the United States.

Norovirus has substantial strain diversity and considerable environmental stability (CDC 2001a). Norovirus does not provide lasting immunity upon infection partly because there are many different serotypes (Carter 2005). Although a small percentage (about 20 percent) of individuals in the United States may be genetically immune to norovirus infection (Lindesmith et al. 2003), the remaining population is subject to repeated episodes of infection and illness. Environmental stability allows norovirus to survive in ground water and elsewhere in the environment until acquired by another host. Individuals can acquire primary norovirus infection by drinking contaminated water or can acquire secondary infection by contact with a contaminated surface or persons.

Norovirus is shed in appreciable numbers, at concentrations similar to enteroviruses (Carter 2005). Because norovirus is highly infectious (Moe et al. 2001), individuals may easily acquire infection. Individuals can easily spread infection and illness to family members and others (both children and adults) outside the household by casual contact with asymptomatic carriers who shed for long periods.

### ***Hepatitis A Virus***

Hepatitis A virus is believed to be highly infectious, perhaps as infectious as norovirus or rotavirus. Hepatitis A (HAV) virus is the only waterborne virus that is reportable to CDC. About 28,000 HAV cases are reported to CDC each year, although that number may decline with time due to new vaccination programs for children. However, because HAV is more severe as an adult disease, an aging U.S. population may have greater disease burden. Mead et al. (1999) estimate about 83,000 HAV cases each year, with a hospitalization rate of 13 percent and a mortality rate of 0.3 percent. Hepatitis remains viable in the environment, especially ground water, for months, and it has a long incubation period. Thus, the infection source is often obscure. Ground water HAV outbreaks have been identified (Georgetown, Texas (Hejkal et al. 1982); Racine, Missouri (Missouri Department of Health 1992); Lancaster, Pennsylvania (Bowen and McCarthy 1983); and Quebec, Canada (De Serres et al. 1999)); all occurred in sensitive aquifers. HAV is difficult to recover in environmental samples because it cannot be easily cultured in the cell lines commonly used in cell culture.

### ***Hepatitis E Virus***

Hepatitis E (HEV) virus is another fecal-oral virus that may be transmitted via ground water. Based on serology and case histories of individual patients, HEV is thought to be endemic in the United States (Tsang et al. 2000). However, the data suggest that only one or a few percent of the population has been infected (Mast et al. 1997). Unlike HAV, no ground water outbreaks of HEV have occurred in the

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<sup>2</sup>Common source outbreaks arise primarily from food or water (ice is considered to be water by EPA but is commonly treated as food in outbreak compilations). Propagation by secondary (person-to-person) transmission may also occur but is not the immediate cause of the outbreak. Outbreaks that are not common source arise and propagate only by person-to-person transmission.

United States, although they have occurred elsewhere (China and Somalia). HEV is not culturable and no data on environmental occurrence in ground water are available.

### ***Adenovirus***

The adenovirus are a large group of viruses that produce diverse symptoms. Two adenovirus serotypes, adenovirus 40 and 41, produce primarily enteric symptoms, but several other adenoviruses are also capable of producing such symptoms. Some cause conjunctivitis. All adenoviruses, no matter the infection site and characteristic illness, are shed through the gut and are thus fecal-oral viruses (Carter 2005). Adenoviruses are not efficiently recovered using commonly available cell lines and methods. Adenovirus is unusual among the viral pathogens because it is capable of causing death in healthy young adults in the United States (CDC 2001b).

### ***Astrovirus***

Astrovirus, like rotavirus, is commonly acquired in child care settings. It causes mild disease in children, and most children are exposed at an early age. However, like rotavirus, a small percentage of that large population suffer more significant health effects and may require in-patient care. Like rotavirus, the disease burden in older children and adult populations is underestimated because the disease is mild (Carter 2005). Astroviruses are shed in stool in large numbers (similar to enteroviruses) and, in France, prospective epidemiology studies have implicated untreated ground water as a route of infection (Gofti-Laroche et al. 2003). Astroviruses are not favored for recovery in environmental samples using common cell lines.

### ***Reovirus***

Reovirus is related to rotavirus and thus has some similar characteristics. Unlike rotavirus, reovirus rarely causes disease, although it is now recognized as a pathogen in children (Tyler et al. 2004). Reovirus is recovered in environmental samples using the buffalo green monkey (BGM) cell line and is commonly found co-occurring with enteroviruses in PWS wells. Although reovirus is probably not a significant component of the total disease burden, it is important because it likely reduces the enterovirus recovery efficiency<sup>3</sup>, which is typically not greater than 50 percent under optimal conditions. Carducci et al. (2002) found that, in some cases, enterovirus detection was limited because reovirus reproduction was so highly favored.

#### **2.2.1.3 Other Pathogens**

Another pathogen that may be found in ground water is the protozoan *Naegleria fowleri*. *N. fowleri* causes primary amebic meningitis, which is difficult to quickly diagnose and treat, and if unsuccessfully treated, is fatal. The young, elderly, and immuno-compromised are especially at risk (Blair and Gerba 2006). Although this organism is usually found in surface water, particularly in tropical or sub-tropical environments, it can also survive in warm groundwater. Most outbreaks occur in recreational waters, but one outbreak associated with drinking water killed two children in 2002 (Marciano-Cabral et al. 2003). *N. fowleri* has three stages in its life cycle. First is the cyst, which can survive in soil for extended periods. Second is the flagellate stage, during which the organism feeds on heterotrophic bacteria living in the groundwater. In the last phase, the organism becomes an ameba or trophozoite, and

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<sup>3</sup>The presence of reovirus in environmental samples interferes with the recovery, or detection and quantification, of enteroviruses. Therefore, reovirus occurrence can cause underestimates of enteroviral occurrence, while elevating overall virus concentrations.

may invade animal tissues. In humans and livestock, if inhaled, it can penetrate the mucus membranes in the nasal passages and reach the brain, causing meningitis. In Arizona, a recent study found fragments of *N. fowleri* DNA in 8.3 percent of the 143 wells tested in alluvial aquifers near Phoenix and Tucson, although samples were examined using the polymerase chain reaction (PCR) method, which does not distinguish between live and dead organisms. Live *N. fowleri* trophozoites were detected in one well (Blair and Gerba 2006).

There may be other as yet undetermined organisms or infectious agents capable of causing waterborne disease. Brainerd diarrhea consists of severe diarrhea lasting more than one year, marked by urgency and fecal incontinence. It may be associated with other microorganisms and agents such as *Campylobacter* bacteria, amoebae, and noroviruses (Kimura et al. 2006, Vugia et al. 2006), but no etiologic agent has been determined. In foodborne and waterborne outbreaks, such as the one described below, there appears to be an association with uncooked liquids and foods, suggesting an infectious agent as the cause. However, treatment with antimicrobial drugs does not eliminate or reduce symptoms, and no new microorganisms or infectious agents have been detected in stool samples or food and water samples (Mintz 2003). In 1996, an outbreak of Brainerd diarrhea associated with a restaurant affected 114 people in Fannin County, Texas (Kimura et al. 2006).

Because pathogens themselves are difficult and expensive to test for, on a day-to-day basis it is more useful to monitor for indicators of fecal contamination rather than pathogens themselves, as described in section 2.2.2 below.

#### **2.2.1.4 Chronic Health Effects Resulting from Waterborne Disease**

In addition to the short-term health effects described in the previous sections, some pathogens are also associated with chronic health effects, as described below.

There is considerable information that Type 1 diabetes may be associated with enterovirus infection, including infection with coxsackievirus and echoviruses (Maria et al. 2005, Vreugdenhil et al. 2000). Epidemiological studies have shown a strong correlation between diabetes and enterovirus infection, and individual case studies have reported diabetes development after enterovirus infection (Roivainen 1998). Recently, Maria et al. (2005) reported simultaneous (on the same day) diabetes onset in mother and son coincident with enteroviral infection. A possible mechanism is that enterovirus infection triggers autoimmunity response (Vreugdenhil et al. 2000).

Enterovirus infection (and other viruses) can sometimes lead to substantial cardiac damage and severe acute heart failure and can also evolve into chronic heart failure (Kearney et al. 2001), although usually viral infections of the heart are mild. Viral infection is the most common cause of myocarditis (inflammation of the heart). Coxsackie B virus is the type of virus most often associated with myocarditis (Kearney et al. 2001). In addition to myocarditis, epidemiological studies from Finland have documented an association between enterovirus infection and heart attacks (myocardial infarction) in men with no prior evidence of heart disease (Reunanen et al. 2002).

The mean age of patients with active myocarditis is 42 years (Kearney et al. 2001). In one study, 60 percent of myocarditis patients had antecedent symptoms indicative of recent viral infection (Kearney et al. 2001). Myocarditis accounted for 22 percent of sudden unexpected deaths under age 30 and 11 percent of those between 30 and 40. Mortality was 20 percent at one year and 56 percent at four years (unless a transplant occurred) largely due to chronic heart failure (dilated cardiomyopathy).

Shiga toxin-producing *E. coli* and other bacteria are also associated with long term effects, particularly kidney problems. Death or end-stage renal disease occurs in about 12 percent of patients four



years after diarrhea-associated kidney failure associated with *E. coli* (Garg et al. 2003). Twenty-five percent of kidney failure survivors demonstrate long-term renal sequelae (Garg et al. 2003). For patients with moderate and severe gastroenteritis caused by *E. coli*, long-term study shows that they have an increased risk of hypertension and reduced kidney function (Garg et al. 2005).

In a Danish study of patients with bacterial foodborne illnesses, the death rate was particularly pronounced during the 30 days after infection, but it remained high up to a year later for those who had been infected with *Salmonella* and *Campylobacter* (Helms et al. 2003). Complications were often due to invasive illnesses occurring within 30 days of infection, including septicemia, endocarditis, pneumonia, and meningitis.

## 2.2.2 Fecal Contamination Indicators

Fecal contamination in ground water can be identified in two ways: 1) monitoring for pathogens, and 2) monitoring for non-pathogenic fecal indicator microorganisms, whose presence suggests fecal contamination and, therefore, the potential presence of pathogens. However, viruses associated with waterborne disease are either difficult to culture (e.g., HAV and rotavirus) or are non-culturable (e.g., norovirus). Some bacteria, such as *Shigella*, are also difficult to culture. Pathogen concentrations in water tend to be low, so the analysis of a large sample volume is required, increasing the analytical costs. Analytical methods for the indicator organisms are typically more widely available, more widely used, and are significantly less expensive than methods for monitoring each of the pathogens.

A variety of candidate indicator microorganisms have been proposed in the scientific literature (Grabow, 2001; for a recent review of the literature, see IAWPRC Study Group, 2001). Based in part on these proposals, a number of indicator microorganisms were selected for sampling by the study investigators as part of the studies reviewed in Chapter 6 of this document. A list of bacterial and viral indicators is presented in Exhibit 2.2.

### Exhibit 2.2 Microbial Indicator Organisms

Waterborne Indicator Bacteria	Waterborne Indicator Viruses
Heterotrophic Plate Count Bacteria (HPC) Total Coliform, Fecal Coliform <i>Escherichia coli</i> ( <i>E. coli</i> ) Fecal Streptococci Enterococci <i>Clostridium perfringens</i> (anaerobic spores) <i>Bacillus subtilis</i> (aerobic spores)	Bacteriophage <i>Bacteroides</i> phage Coliphage Male-specific coliphage F+RNA phage F+DNA phage Somatic coliphage

Source: Adapted from Karim et al. 2002; USEPA 2001a; Lederberg 1992; Stewart 1998; Lieberman 2002; and Abbaszadegan 1999.

Three indicators, *E. coli*, enterococci, and coliphage, were chosen as indicators for the GWR (use of EPA-approved methods is required). EPA believes that these three indicators are closely associated with fecal contamination. Of the three types of fecal indicators specified in the GWR, EPA does not have a single preferred choice of indicator because no single indicator can definitively determine whether pathogens are present. States should refer to the *Source Water Monitoring Guidance* (USEPA, forthcoming), for guidance on when coliphage monitoring may be more appropriate.

### 2.2.2.1 Bacterial Indicators

Bacteria that have been used as indicators of fecal contamination include the total coliform (TC) bacterial species, many of which are free-living in the environment, and fecal bacteria including *E. coli*. Fecal coliforms are bacteria found in animal feces. Because TC bacteria are primarily free-living in the environment, when identified at the tap they are considered to be indicators of chlorine demand and distribution system contamination as well as possible fecal contamination of source water. When TC are identified in source ground water they may be indicators of surface or near-surface inflow to ground water as well as possible fecal contamination. Other bacteria that are used as indicators of fecal contamination include the fecal streptococci (enterococci) and *Clostridium perfringens*, a spore-forming anaerobic organism. Some indicator bacteria have specialized uses. For example, heterotrophic plate count (HPC) bacteria may be used to track treatment efficiency, and *Bacillus* is used as an indicator of surface or near-surface water inflow to ground water (Rice 1996). Both HPC and TC bacteria are used to identify the presence of biofilm or other distribution system problems (Geldreich 1996, Carter et al. 2000).

Bacterial indicators such as TC and fecal coliform have, in the past, been considered to originate in the intestines of warm-blooded animals, including humans. However, more recent work by Conboy and Goss (2001) reported that fecal material from 28 different animals, including reptiles, contained TCs, fecal coliform, and fecal streptococci. *Clostridium perfringens* was found in all but two of those animals.

### 2.2.2.2 Viral Indicators

Bacteriophages (“phages”) are viruses that infect bacteria. They can replicate only in a living host bacterial cell. Some phages are considered to be indicators of human enteric viruses (Curry 1999; Grabow 2001). Phages commonly used as models/surrogates in water quality assessments include the coliphage group (phages that infect *E. coli*). They are good indicators for a number of reasons:

- Are similar to human enteric viruses in shape, size, morphology, and composition (using electron microscopy, F+RNA coliphages and enteroviruses are almost indistinguishable (Grabow 2001))
- Respond to water treatment and natural environments similarly to human enteric viruses
- May be similar to the enteric viruses in transport efficiency through soil and aquifer materials due to similar size and shape
- It is unlikely that they can reproduce in water environments due to strict conditions (e.g., temperature, log phase growth of their hosts) needed for replication (Grabow 2001)

Receptor sites for coliphages on bacterial hosts are located on either the cell wall (somatic coliphage) or on the fertility fimbriae (male-specific coliphage) in certain strains of *E. coli*. In *E. coli*, fimbriae (or pili) are generally only produced at temperatures in the range of 30-45 degrees C, the temperature of the human gut, and only by bacteria in the log-growth phase. The pilus allows for transfer of nucleic acid from one bacterium to another. Male-specific coliphages (F+) may have either RNA (F+RNA phages) or DNA (F+DNA phages) as their genetic material (Grabow 2001).

Some bacteriophages are more frequently associated with fecal contamination than others. Coliphages are bacteriophages that primarily infect and replicate in the bacterium *E. coli* and appear to be

present wherever *E. coli* are found. Feces-specific bacteriophage, including the male-specific F+RNA coliphages, somatic coliphages, and *Bacteroides fragilis* bacteriophages, are described below. It should be noted that *B. fragilis* is not widely used as an indicator in the United States because counts are usually low in this country, so it is not considered a useful indicator for U.S. waters (Stewart 1998). Gantzer et al. (2002) found that somatic coliphages occurred in 68 percent of human stool samples in eastern France at a mean concentration of  $4.3 \times 10^3$  plaque-forming units per gram (PFU/g) and *Bacteroides fragilis* phages in 11 percent of samples at a mean concentration of  $7 \times 10^1$  PFU/g.

Host-range data indicate the fecal origin of most male-specific and somatic coliphages (Stewart 1998). However, about 15 percent of 485 somatic coliphage samples assayed showed that somatic coliphages also infect bacteria that normally live in the soil, or ground water (Stewart 1998), and thus are not fecal indicators. F+RNA prefer high temperatures (i.e., over 30 degrees C), but F+DNA bacteriophages and somatic *Salmonella* bacteriophages grow well at both high and low temperatures (Stewart 1998). Thus, male-specific coliphages may be somewhat better fecal indicators than somatic coliphages because male-specific coliphages are found more frequently at temperatures comparable to the human gut.

The occurrence of F+RNA and F+DNA male-specific and somatic coliphages in septic tanks has been investigated by DeBorde (1998, 1999). DeBorde conducted one-time sampling for coliphages in 100 septic tanks and quarterly sampling of 10 septic tanks. The analyses show that coliphages occur in less than one-half of septic tanks at any time. Somatic coliphages were found at the greater frequency, as compared with male-specific coliphages.

Human enteric viruses and coliphages are not excreted in the same numbers, or for the same time periods. Therefore, direct correlations between the numbers of coliphages and enteric viruses in water environments are not possible (Grabow 2001). Grabow (2001) found that coliphages are excreted at all times by some mammal populations, whereas human enteric viruses are excreted only for a few days to a few weeks. Male-specific coliphages are shed by a small number of people (13, or about 2 percent in one study) (Osawa 1981), regardless of whether enteric viruses are present.

Grabow estimates the following regarding the relative occurrence of coliphages in water environments (Grabow 2001; Stewart 1998):

- Somatic coliphages may replicate faster than male-specific coliphages by a factor of five
- Somatic coliphage may outnumber assayable human viruses by a factor of 500.
- Male-specific coliphages found in wastewater and raw water were 100 times more prevalent than pathogenic enteric viruses.

However, Grabow's estimates may not be typical of U.S. wastewater because concentrations are affected by the overall quality of public health or animal populations.

While phages can be viable and culturable in water environments, it is unlikely that they can reproduce in water environments due to strict conditions (e.g., temperature, log phase growth of their hosts) needed for replication. Conditions for growth generally do not exist in surface water, or even in sewage, so it is unlikely that male-specific coliphages will replicate in groundwater. However, Grabow (2001) reported studies where counts of somatic coliphages increased in sand filters and biologically active carbon filters. Bacterial hosts may metabolize or even multiply in filters so that virus replication is possible, but not likely to occur in large numbers (Grabow 2001, Stewart 1998).

Some bacteriophages convey new properties to their host bacterial cells, and induce the normally harmless bacteria to become toxin-producing pathogens (e.g., *E. coli* O157:H7). For this reason, it can be argued that the absence of phages from PWS sources is as desirable as the absence of human pathogenic viruses (Grabow 2001).

Coliphage hosts and other phage hosts have similar receptors. Phages other than coliphages can bind to these receptors. Thus, bacteriophages that infect these other hosts may sometimes infect *E. coli*, and coliphages may infect other bacteria besides *E. coli*. Therefore, laboratory efforts to quantify coliphages are often confounded by bacteriophages different from those of concern (Stewart 1998). For this reason, host strains used for detecting male-specific coliphage include *S. typhimurium* (WG49) because it is not susceptible to somatic coliphages that tend to interfere with detection of male-specific coliphages when *E. coli* hosts are used. Grabow (2001) concluded that phages are best applied as models/surrogates in controlled laboratory experiments rather than in natural water environments.

Studies describing co-occurrence of phages with bacteria in different water environments are provided in Chapter 6.

### **2.2.3 Bacterial Host Range for Bacteriophage Indicators of Fecal Contamination**

Host-specificity of phages is a useful way of classifying bacteria, and discussion of these is useful in interpreting occurrence data. As described in the previous section, phages are selective in their preferred species of bacteria. They are also selective for individual strains of a given bacteria species. As an example, coliphages prefer *E. coli* but may infect other bacteria, as described below. *E. coli* O157:H7 can be further divided into 66 strains based on the phages that infect it. Also, *E. coli* strain C (also known as WG4) is a mutant strain that is susceptible to a wide range of coliphages and is the most commonly used host for detecting somatic coliphages in water environments (Grabow 2001).

In addition to *E. coli*, some male-specific and somatic coliphages are capable of infecting *Klebsiella pneumoniae* and *Enterobacter cloacae*, both members of the coliform group. They also infect *Pseudomonas aeruginosa* (Stewart 1998). These coliform bacteria are rarely found in the mammalian gut and, when present, are found in low numbers. They are usually found free-living in the environment. According to Geldreich (1996), *Klebsiella*, *Enterobacter*, and *Nitrobacter* are found most commonly as environmental, rather than fecal, organisms. That is, they originate outside the mammalian gut. Although some *Klebsiella* strains can be of fecal origin, the vast majority of *Klebsiella* encountered in drinking water are environmental strains that inhabit vegetation and agricultural or wood products.

More information about the pathogens of concern and their indicator microorganisms may be found in three EPA drinking water criteria documents for viruses (USEPA 1985a; 1999a; 1999b) and three EPA criteria documents for bacteria (USEPA 1984a, b; 1985c). Leclerc et al. (2000) and Grabow et al. (2001) have published recent reviews on the use of bacteriophage as indicator organisms for ground water.

## **2.3 Waterborne Disease Outbreaks**

This section presents a detailed review of waterborne disease outbreaks associated with ground water sources. Outbreak characterization is useful for indicating relative degrees of risk associated with different types of source water and systems.

The CDC maintains a database of information on waterborne disease outbreaks in the United States. The database is based upon responses to a voluntary and confidential survey form that is completed by state and local public health officials. CDC defines a waterborne disease outbreak as occurring when at least two people (one person for chemical exposure) experience a similar illness after ingesting a specific drinking water (Kramer et al. 1996). Data from the CDC summary reports and database appear in Exhibits 2.3 and 2.4, presented later in this section.

The U.S. National Research Council suggested that the number of identified and reported outbreaks in the CDC database (both for surface and ground waters) represents a small percentage of actual waterborne disease outbreaks (National Research Council 1997). In practice, most waterborne outbreaks in CWSs are not recognized by officials until a sizable proportion of the population is ill (Perz et al. 1998; Craun 1996), perhaps 1 to 2 percent of the population (Craun 1996). Some of the reasons for the lack of recognition and reporting of outbreaks, most of which were noted by the National Research Council (1997) and Frost et al. (1996), are as follows:

- Some states do not have active disease surveillance systems. Thus, states that report the most outbreaks may not be those in which the most outbreaks occur.
- Even in states with effective disease surveillance systems, health officials may not recognize the occurrence of small outbreaks. In cities, large outbreaks are more likely to be recognized than sporadic cases or small outbreaks in which ill people may consult different physicians. Even so, health authorities did not recognize the massive outbreak (403,000 illnesses) of waterborne cryptosporidiosis that occurred in Milwaukee, Wisconsin, in 1993 until the disease incidence was near or at its peak (McKenzie et al. 1994). The outbreak was recognized when a pharmacist observed that the sale of over-the-counter diarrheal medicine was very high and consequently notified health authorities.
- Most cases of waterborne disease are characterized by general symptoms (diarrhea, vomiting, etc.) that cannot be distinguished from those caused by other disease sources (e.g., foodborne outbreaks).
- Only a small fraction of people who develop diarrheal illness seek medical assistance.
- Many public health care providers may not have sufficient information to request the appropriate clinical test.
- If a clinical test is ordered, the patient must comply, a laboratory must be available and proficient, and a positive result must be reported in a timely manner to the health agency.
- Not all outbreaks are effectively investigated. Outbreaks are included in the CDC database only if water quality and/or epidemiological data are collected to document that drinking water was the route of disease transmission. Monitoring conducted after the recognition of an outbreak may be too late to detect an intermittent or a one-time contamination event.
- Some states do not always report identified waterborne disease outbreaks to the CDC. Reporting outbreaks is voluntary.
- The vast majority of ground water systems are NCWSs. Outbreaks associated with NCWSs are less likely to be recognized than those in CWSs because NCWSs generally serve non-residential areas and transient populations.

For all of the above reasons, the aggregate outbreak data are probably not representative of the actual occurrence of microbiological contamination. There is also the issue of endemic waterborne disease. Endemic waterborne disease may be defined as any waterborne disease not associated with an outbreak, or more precisely, as the normal level of waterborne disease in a community. Under this definition, an outbreak would represent a spike in the incidence of disease. Based on this definition, the level of endemic waterborne disease in a community may be quite high. For example, 14 to 40 percent of the normal gastrointestinal illness in a community in Quebec was associated with treated drinking water from a surface water source (Payment et al. 1997). In principle, high numbers of endemic disease could also be associated with ground water sources, and the national numbers of endemic waterborne disease are likely to be much higher than the numbers of disease associated with reported outbreaks. However, EPA is not aware of any data on the national or local incidence of waterborne endemic disease, especially in ground water systems, and consequently, endemic disease numbers (from all waterborne pathogens) were not used directly in assessing risk in developing the GWR. Instead, endemic disease numbers attributable to ground water sources were modeled based on best information available from viral pathogen occurrence studies. The *Economic Analysis for the Final Ground Water Rule* (USEPA 2006a) provides a detailed description of the model and input parameter values.

Exhibits 2.3 and 2.4 present outbreak data only for CWSs and NCWSs using ground water as a source from 1991 to 2000. These data more accurately represent the occurrence of outbreaks since the implementation of current drinking water regulations (e.g., the Total Coliform Rule). The data do not include outbreaks caused by chemical contamination, since the GWR only regulates microbial contamination. The data do include outbreaks caused by protozoans such as *Giardia* and *Cryptosporidium* in ground water systems, even though such systems are considered to be ground water under the direct influence of surface water (GWUDI). GWUDI systems are subject to regulation as surface water systems. It is not uncommon for ground water PWSs, regulated only by the TCR, to be found to be contaminated with *Giardia* and *Cryptosporidium* (e.g., Brushy Creek, Texas, and South Bass Island, Ohio (Bergmire-Sweat 1999, Ohio EPA 2005)). In these cases, the system is retrospectively recognized after the outbreak to be a GWUDI system rather than a ground water system. Exhibit 2.4 shows causes associated with waterborne disease outbreaks and illnesses in ground water systems. The outbreak data indicate that the major deficiency in ground water systems was source water contamination—either untreated or inadequately disinfected ground water. Contaminated source water was the cause of more than 79 percent of the outbreaks in ground water systems, accounting for approximately 63 percent of the outbreaks in CWSs and 86 percent in NCWSs.

## Exhibit 2.3 Sources of Waterborne Disease Outbreaks in Ground Water Systems, 1991-2000\*

Cause of Contamination	Number of Outbreaks	Percent Outbreaks	Cases of Illness	Percent Illnesses	Cases per Outbreak
<b>Community Water Systems</b>					
Untreated Ground Water	5	26%	167	6%	33
Treatment Deficiency	7	37%	1,624	58%	232
Distribution System Deficiency	5	26%	803	29%	161
Miscellaneous/Unknown	2	11%	183	7%	92
<b>Total</b>	<b>19</b>	<b>100%</b>	<b>2,777</b>	<b>100%</b>	<b>146</b>
<b>Noncommunity Water Systems</b>					
Untreated Ground Water	23	47%	4,057	50%	176
Treatment Deficiency	19	39%	3,264	40%	172
Distribution System Deficiency	6	12%	442	5%	74
Miscellaneous/Unknown	1	2%	386	5%	386
<b>Total</b>	<b>49</b>	<b>100%</b>	<b>8,149</b>	<b>100%</b>	<b>166</b>
<b>Combined</b>					
Untreated Ground Water	28	41%	4,224	39%	151
Treatment Deficiency	26	38%	4,888	45%	188
Distribution System Deficiency	11	16%	1,245	11%	113
Miscellaneous/Unknown	3	4%	569	5%	190
<b>Total</b>	<b>68</b>	<b>100%</b>	<b>10,926</b>	<b>100%</b>	<b>161</b>

Sources: CDC (1993), Kramer et al. (1996), Levy et al. (1998), Barwick et al. (2000), and Lee et al. (2002)

Exhibit 2.4 identifies the etiology of waterborne outbreaks in ground water systems. Of the 68 outbreaks in ground water systems, 14 (20.6 percent) were associated with specific bacterial pathogens. Etiologic agents were not identified in 39 (57.4 percent) of the outbreaks. The diversity of disease agents is greater than that of surface water, which are dominated by protozoa and viruses. EPA suspects that many, perhaps a majority, of the outbreaks where an agent was not determined were virus-caused, given the fact that it is generally more difficult to analyze for viral pathogens than bacterial pathogens. Recent improvements in analytical methods for viruses suggest that noroviruses are responsible for a larger percentage of outbreaks, although it is also possible that the number of norovirus outbreaks is increasing (Widdowson et al. 2005). The protozoan pathogen, *Giardia*, and the fecal bacterial pathogen, *Shigella*, caused 10.3 and 7.4 percent of reported outbreaks, respectively, more than any other agents.

Collectively, the data indicate that outbreaks in ground water systems are a problem and that source contamination and inadequate treatment (or treatment failures) are responsible for the great majority of outbreaks. The outbreaks are caused by a variety of pathogens, most of which cause short-term gastrointestinal disease.

## Exhibit 2.4 Etiology of Waterborne Outbreaks in Ground Water Systems, 1991-2000

Causative Agent	CWSs			NCWSs			TOTAL		
	Outbreaks	Cases of Illness	Percent of Total Outbreaks	Outbreaks	Cases of Illness	Percent of Total Outbreaks	Outbreaks	Cases of Illness	Percent of Total Outbreaks
<b>Protozoa</b>	<b>8</b>	<b>1,675</b>	<b>42.1%</b>	<b>3</b>	<b>576</b>	<b>6.1%</b>	<b>11</b>	<b>2,251</b>	<b>16.2%</b>
<i>Giardia</i>	5	136	26.3%	2	25	4.1%	7	161	10.3%
<i>Cryptosporidium</i>	3	1,539	15.8%	1	551	2.0%	4	2,090	5.9%
<b>Virus</b>	<b>-</b>	<b>-</b>	<b>0.0%</b>	<b>4</b>	<b>1,806</b>	<b>8.2%</b>	<b>4</b>	<b>1,806</b>	<b>5.9%</b>
Hepatitis A	-	-	0.0%	-	-	0.0%	-	-	0.0%
Norwalk Virus	-	-	0.0%	4	1,806	8.2%	4	1,806	5.9%
<b>Bacteria</b>	<b>6</b>	<b>1,037</b>	<b>31.6%</b>	<b>8</b>	<b>1,309</b>	<b>16.3%</b>	<b>14</b>	<b>2,346</b>	<b>20.6%</b>
<i>Shigella</i>	1	83	5.3%	4	473	8.2%	5	556	7.4%
<i>Campylobacter</i>	1	172	5.3%	2	51	4.1%	3	223	4.4%
<i>Salmonella</i> , non-typhoid	1	625	5.3%	-	-	0.0%	1	625	1.5%
<i>S. typhimurium</i>	1	124	5.3%	-	-	0.0%	1	124	1.5%
<i>E. coli</i>	1	22	5.3%	2	785	4.1%	3	807	4.4%
<i>Vibrio</i>	1	11	5.3%	-	-	0.0%	1	11	1.5%
<b>Undetermined</b>	<b>5</b>	<b>65</b>	<b>26.3%</b>	<b>34</b>	<b>4,458</b>	<b>69.4%</b>	<b>39</b>	<b>4,523</b>	<b>57.4%</b>
<b>Total</b>	<b>19</b>	<b>2,777</b>	<b>100.0%</b>	<b>49</b>	<b>8,149</b>	<b>100.0%</b>	<b>68</b>	<b>10,926</b>	<b>100.0%</b>

Note: Detail may not add to totals due to rounding.

Sources: CDC (1993), Kramer et al. (1996), Levy et al. (1998), Barwick et al. (2000), and Lee et al. (2002).

### 2.4 Total Coliform-Positive Samples and TCR Violations in Ground Water Systems

This section describes the available data on indicator occurrence based on existing PWS monitoring requirements. The TCR was promulgated in June 1989 and applies to all PWSs. As mentioned previously, TCs are a group of closely related bacteria that are generally free-living in the environment, but are also normally present in water contaminated with human and animal feces. TCs generally do not cause disease (with a few exceptions, as discussed in section 2.2.1.1). They are not used as fecal indicators; rather, they serve as an indicator of treatment effectiveness and of problems in the distribution system. In an untreated or insufficiently treated ground water system, TC can also suggest source water problems. The TCR requires systems to monitor their distribution systems for TC bacteria.

Under the TCR, a system that collects 40 or more samples per month (generally systems that serve more than 3,300 people) is in violation of the maximum contaminant level (MCL) if more than 5 percent of the samples (routine or repeat samples) it collects per month are TC-positive. A system that collects fewer than 40 samples per month is in violation of the MCL if two consecutive samples (routine or repeat) are TC-positive. These are not, however, acute TCR violations. If a system has a TC-positive sample, it must test that sample for the presence of fecal coliform bacteria or *E. coli*. If two consecutive TC-positive samples occur at a site, and one is fecal coliform or *E. coli*-positive, the system has an acute violation of the MCL under the TCR.

Exhibit 2.5 shows the numbers and percentages of systems that violated the monthly or acute MCL. Together, these data allow evaluation of the current status of and public health risk associated with microbial contamination occurrence in ground water. The most recent data suggest that only a very small percentage of ground water PWSs (<1 percent in most system size categories) have acute TCR violations. In the years just after the TCR rule was being implemented, the percentage of acute TCR violations was higher (<3.5 percent). Inspection of the most recent data suggests that, each year, only a small percentage of ground water PWSs (in most cases, less 6 percent of each system size category) have non-acute TCR MCL violations.



## Exhibit 2.5 Numbers and Percentages of Ground Water Systems with Monthly and Acute TCR MCL Violations

	System Size (Population Served)	Number of Systems				Percentage of Systems with Acute MCL Violations as a Percent of Total Systems	Percentage of Systems with Any MCL Violations as a Percent of Total Systems	Mean Percentage of Systems with MCL Violations as a Percent of Total Systems
		Total Systems	Systems with Acute MCL Violations	Additional Systems with Monthly MCL Violations	Total Systems with MCL Violations			
	A	B	C	D	E = B/A	F = (B+C)/A	G = (E+F)/2	
CWS	<100	12,506	46	625	671	0.37%	5.37%	2.87%
	101-500	13,306	56	596	652	0.42%	4.90%	2.66%
	501-1,000	4,233	13	131	144	0.31%	3.40%	1.85%
	1,001-3,300	5,359	10	219	229	0.19%	4.27%	2.23%
	3,301-10,000	2,513	10	155	165	0.40%	6.57%	3.48%
	10,001-50,000	1,233	6	72	78	0.49%	6.33%	3.41%
	50,001-100K	137	0	5	5	0.00%	3.65%	1.82%
	100,001- 1 Million	64	1	2	3	1.56%	4.69%	3.13%
> 1 Million	3	0	0	0	0.00%	0.00%	0.00%	
NTNCWS	<100	9,432	47	442	489	0.50%	5.18%	2.84%
	101-500	6,726	28	246	274	0.42%	4.07%	2.25%
	501-1,000	1,884	6	58	64	0.32%	3.40%	1.86%
	1,001-3,300	706	3	27	30	0.42%	4.25%	2.34%
	3,301-10,000	68	0	3	3	0.00%	4.41%	2.21%
	10,001-50,000	7	0	0	0	0.00%	0.00%	0.00%
	50,001-100K	1	0	0	0	0.00%	0.00%	0.00%
	100,001- 1 Million	1	0	0	0	0.00%	0.00%	0.00%
> 1 Million	0	0	0	0	0.00%	0.00%	0.00%	
TNCWS	<100	63,856	292	2390	2682	0.46%	4.20%	2.33%
	101-500	18,915	106	696	802	0.56%	4.24%	2.40%
	501-1,000	1,913	8	72	80	0.42%	4.18%	2.30%
	1,001-3,300	571	2	37	39	0.35%	6.83%	3.59%
	3,301-10,000	71	0	4	4	0.00%	5.63%	2.82%
	10,001-50,000	17	0	1	1	0.00%	5.88%	2.94%
	50,001-100K	0	0	0	0	0.00%	0.00%	0.00%
	100,001- 1 Million	1	0	0	0	0.00%	0.00%	0.00%
> 1 Million	0	0	0	0	0.00%	0.00%	0.00%	

Source: From SDWIS (USEPA 2003).

To get a full idea of water quality problems in ground water systems nationwide, EPA estimated the percentage and number of samples that are TC-positive in undisinfected ground water systems based on State information. State files contain water systems' reports on the numbers of total coliform samples taken to comply with the TCR and the numbers of TC-positive samples. TC-positive samples are recorded by States but are not reported to EPA unless an MCL violation has occurred.

EPA obtained State information as part of its data verification (DV) efforts; EPA compared data from EPA's Safe Drinking Water Information System (SDWIS) (USEPA 2003) with States' files through site visits to State offices. The DV study involves the comparison of 1 year's worth of PWS records in SDWIS with State PWS records to identify any discrepancies between the two records. Using these data, EPA derived national estimates of the percentage of all TCR samples that test positive for total coliform, and the number of total coliform samples per year that test positive for all systems in the eight categories. The number of samples that test positive for year is weighted by the number of systems in each size category. See Chapter 4 of the *Economic Analysis for the Final Ground Water Rule* (EPA 2006a) for

more details on this analysis. Exhibit 2.6 shows the percentage of samples that are positive for systems serving 1,000 or fewer people and those serving more than 1,000 people, by system type (CWS, NTNCWS, and TNCWS). Exhibit 2.7 shows the number of TC samples each system takes per year, based on TCR requirements (sampling requirements vary with system size, but systems may take more samples than required). Exhibit 2.8 shows the estimated number of positive samples per system per year, determined by multiplying the positive rate in Exhibit 2.6 by the number of samples per system in Exhibit 2.7.

### Exhibit 2.6 Total Coliform-Positive Hit Rates

Type of System	Size of System (Population Served)	TC-Positive Hit Rate (per sample)
CWS	≤1,000	2.72%
	>1,000	0.71%
NTNCWS	≤1,000	2.98%
	>1,000	2.25%
TNCWS	≤1,000	6.36%
	>1,000	3.53%

Source: Chapter 4, *Economic Analysis for the Final Ground Water Rule* (EPA 2006a)

### Exhibit 2.7 Estimated Number of Routine Total Coliform Samples Taken Per System, Per Year, by Type and Size of System

System Type	Population Served	TCR Baseline Number of Routine Samples per System	Estimated Actual Number of Routine Samples per System
CWS	<100	12	14
	101-500	12	15
	500-1K	12	18
NTNCWS + TNCWS	<100	4	7
	101-500	4	8
	500-1K	4	9
CWS + NTNCWS + TNCWS	1011-3300	24	31
	3301-10K	84	82
	10,001-50K	360	311
	50,001-100K	960	924
	>100,001	2,520	1,496

Source: Chapter 4, *Economic Analysis for the Final Ground Water Rule* (EPA 2006a)

## Exhibit 2.8 Estimated Number of TC-Positive Samples Per System, Per Year, by System Size and System Type

System Type	System Size (Population Served)							
	<100	101-500	501-1K	1,001-3,300	3,301-10K	10,001-50K	50,001-100K	>100K
CWS	0.38	0.41	0.49	0.22	0.58	2.2	6.6	10.6
NTNCWS	0.22	0.23	0.28	0.70	1.8	7.0	20.8	33.7
TNCWS	0.47	0.48	0.60	1.1	2.9	11.0	32.6	52.8

Source: Chapter 4, *Economic Analysis for the Final Ground Water Rule* (EPA 2006a)

## 2.5 Conclusions

The number of reported illnesses (from both waterborne disease outbreaks and from endemic waterborne disease) affecting ground water consumers supplied by PWSs appears to be small. However, the limitations associated with current methods for identifying and reporting of outbreaks suggest that a significant number of illnesses occur but are not recognized or reported. Despite the well-recognized difficulties in identifying waterborne disease outbreaks, outbreaks continue to occur and are reported (when recognized) each year. Furthermore, TCR acute violation data suggest that fecal contamination occurs at a small percentage of ground water PWSs. These data suggest that the occurrence of pathogenic and indicator microorganisms is continuing in a small number of systems. Regulatory measures are necessary to protect public health from waterborne disease outbreaks and from endemic illness resulting from consumption of ground water contaminated by viruses or bacteria from fecal sources.



## 3.0 Sources of Fecal Contamination of Ground Water and Wells

### 3.1 Background

This section describes the sources of viral and bacterial fecal contamination that may affect ground water sources and public water system (PWS) wells. Fecal contamination of ground water can be associated with human waste, animal waste, or sources contaminated by such wastes (e.g., stormwater runoff). All types of sewage and solid waste may carry enteric pathogens. In general, bacterial pathogens are generated by both animal and human sources. Viral pathogens that infect humans, in contrast, are typically generated only by human sources. Viruses that infect the animal gut normally do not cause illness in humans, although animal reservoirs for a few enteric viruses are suspected. (Viruses that infect the respiratory system, such as the sin nombre virus, hantavirus, influenza virus, and Ebola virus, may be more likely to have animal reservoirs.) Because enteric pathogens are associated with human and animal waste, the following discussions do not focus on the specific pathogen types associated with each source, but on fecal contamination in general. EPA assumes that local fecal contamination sources may contain any or most of the fecal indicator organisms or waterborne pathogens.

Potential sources of human fecal contamination that can infiltrate and contaminate ground water include:

- Improperly located, designed, constructed, operated, or maintained septic systems
- Open sewage ponds
- Inadequately treated sewage treatment plant effluent used to irrigate crop land
- Unlined or leaking sewage treatment plant lagoons
- Land application of improperly treated biosolids (sewage treatment plant residue)
- Ruptured, leaking, or overflowing sewer collection lines
- Combined sewer overflow (CSO) (untreated sewage mixed with stormwater)

Furthermore, solid wastes contaminated with human bacteria and viruses may contaminate ground water through individual waste disposal practices, open dumping practices, and landfills and landfill leachate.

Major sources of animal fecal contamination that can reach ground water include:

- Leakage or overflow from manure storage piles or lagoons at animal feeding operations (AFOs) (feedlots) and concentrated animal feeding operations (CAFOs)
- Land application of improperly treated wastewaters associated with food processing or animal slaughter
- Animal wastes from pets, animal husbandry, or wild animals

In the following sections, occurrence data from the fecal contamination sources that present the greatest hazard (high density contamination disposed to the land surface or injected into the shallow subsurface) are discussed in detail. This discussion is accompanied by information on PWS waterborne disease outbreaks linked to or associated with these sources, where such information is available. EPA recognizes that the promulgation and implementation of the Total Coliform Rule (TCR) has enhanced drinking water quality and that circumstances leading to outbreaks that occurred prior to about 1990 might not lead to outbreaks if they occurred today, given the public health improvements embodied in the TCR and other drinking water protection activities.

### **3.2 On-Site Wastewater Systems (Septic Tanks and Drainfields)**

On-site wastewater systems typically consist of a concrete tank, which performs best under anaerobic conditions, and an associated piped drainfield system, which functions under aerobic unsaturated conditions. The tank is designed to slow down the movement of septage and to promote the removal of solids either by settling or by liquefaction. There is a single intake pipe and multiple, distributed egress pipes that discharge effluent to the shallow soil and are designed to maximize soil treatment of microbial contamination and prevent human contact with septage. When properly located, designed, constructed, operated, and maintained, on-site sewage systems are effective in preventing fecal contamination of PWS wells because microbial contaminants become inactivated or are removed by the soil.

Overflow or seepage from improperly located, designed, constructed, operated, or maintained septic tanks, drainfields, and cesspools (community cesspools are no longer permitted in all states, but individual cesspools continue to be used) are among the most frequently reported sources of ground water contamination linked to disease outbreaks in the United States (Yates et al. 1985). Fecal contamination of untreated ground water continues to be a major cause of outbreaks (Blackburn et al. 2004). Although updated estimates on the association between onsite wastewater systems and outbreaks are not available, onsite wastewater treatment systems are probable sources, as described in the studies below. Many existing onsite wastewater systems were constructed years ago, when design and siting guidelines were less robust. In addition, many existing drinking water wells may also not be sited optimally.

The occurrence of F+RNA and F+DNA male-specific and somatic coliphage (see Chapter 2) in septic tanks has been investigated by DeBorde (1998, 1999). DeBorde conducted one-time sampling of 100 septic tanks for coliphage and quarterly sampling of 10 septic tanks. The samples show that coliphage occur in less than one-half of septic tanks at any one time.

Yeager and O'Brien (1977) estimated the average number of enteric viruses present in septic tank effluent to be  $2.5 \times 10^6$  plaque-forming units per liter (PFU/L), while in 1979, Hain and O'Brien estimated this number to be  $3.2 \times 10^3$  PFU/L. More recently, Deborde et al. (1998) calculated septic effluent enteric virus concentrations (calculated by the most probable number (MPN) method) of 4.4 and 0.26 virus/L. Only 2 of 16 samples showed detectable enteric virus concentrations. DeBorde et al. write that the measured values were lower than anticipated, based on the large number of fecal waste contributors (350 people). Hagedorn (1984) reported bacterial density in septic tanks (average of five tanks) as: total coliform (TC) - 3.4 million per 100 milliliters (mL), fecal coliform - 420,000 per 100 mL, fecal streptococci - 3,800 per 100 mL, and *Pseudomonas aeruginosa* 10,000 per 100 mL.

Anderson et al. (1991) reported that monitoring wells located a few feet downgradient of septic systems in residential areas indicated evidence of fecal contamination (coxsackie B4 virus) of ground water 2 to 3 feet directly below the bottom of the septic system drainfield bed in fine sandy soils. Ground

water from a domestic well completed in the same formation, but 10 feet further downgradient of the infiltration bed, was negative for enteric viruses during the year long study. Alhajjar et al. (1988) investigated the movement of bacteria and viruses from septic tanks in Wisconsin. Bacteria were not found in the ground water, but poliovirus was found to have transported to ground water and to a lateral distance of 6 meters (m) within the ground water. The Desert Water Agency (1993) investigated the ground water beneath a high-density region of septic tanks in Cathedral City, California. Five monitoring wells located within the community and surrounded by septic tanks (the PWS wells are located four miles away) were positive for coliphage. Two monitoring wells were positive for enteric viruses.

Curry (1999) and the New York Department of Environmental Protection designed and installed residential septic tank systems at six sites. Monitoring wells for each site included either one or two upgradient wells and up to eight downgradient wells. Monitoring was conducted for up to 77 days per well for male-specific coliphage and *Salmonella* PRD-1 phage, as well as *B. subtilis* that had been added to the distribution boxes of each of the septic systems. Positive samples were found for up to 62 days. PRD-1 was found at four of the six sites. MS-2 and *B. subtilis* were found at five of the six sites. The maximum distances for positive samples at each of the six sites was greater than 100 feet.

Septic systems that serve multiple dwellings are particularly of concern because failure can result in high volumes of concentrated sewage entering ground water. EPA regulates multiple dwelling, community, and regional septic systems (as opposed to individual or single family residential septic systems) as “Class V injection wells” as part of its Underground Injection Control program. Such wells may also include shallow domestic wastewater treatment plant effluent disposal wells (40 CFR 144.1(g)(1)-(2)). EPA prohibits underground injection if injection has the potential to contaminate an underground source of drinking water, causing illness or a violation of drinking water standards.

Disease outbreaks have been linked to septic system contamination of drinking water supply wells. Several of these outbreaks are listed in Exhibit 3.1 and described further in the text that follows. In many of the outbreaks, no etiologic agent was detected in water, blood, or stool samples due to lack of testing or difficulty of detection, but it is possible that viruses may have caused the outbreaks.

### Exhibit 3.1 PWS Waterborne Disease Outbreaks Associated with On-Site Wastewater Systems

Outbreak Summary	Pathogen and Concentration in Well Water (If Identified)
Hepatitis A in a church camp in Racine, MO	Not available
Hepatitis A in a migratory worker camp in Homestead, FL	Echovirus (1 PFU/100 gallons)
Shigellosis in Richmond Heights, FL	Not available
Gastroenteritis in a restaurant in MI	Not available
Gastroenteritis in a campground in SD	Poliovirus (vaccine strain) (1PFU/5 gallons)
Gastroenteritis in restaurant in Yukon, Canada	Not available
Hepatitis A in Buttermilk Falls spring, KY	Not available
Gastroenteritis, resort in Drummond Island, MI	Not available
Gastroenteritis, camp in Coeur d'Alene, ID	<i>Arcobacter butzleri</i>
Gastroenteritis, lodge in Sheridan County, WY	Norovirus
Gastroenteritis, in a restaurant in Central WY	Norovirus
Gastroenteritis, South Bass Island, OH	<i>Arcobacter butzleri</i> , <i>Campylobacter</i> , <i>Salmonella</i> sp., enterovirus, adenovirus

**1. Hepatitis A, Church Camp near Racine, Missouri**—Consumption of water from the two wells supplying a church camp in Racine, Missouri led to a 28-case outbreak of the hepatitis A virus (HAV) among water consumers. The addition of a dye to the septic systems revealed subsurface movement to the church wells within 5 days (Missouri Department of Natural Resources 1992). The camp is located in a karst limestone region.

**2. Hepatitis A, Migratory Worker Camp in Florida**—In late March 1975, an enteric virus disease outbreak, thought to be waterborne, occurred in a migrant labor camp in Homestead, Florida (Wellings et al. 1977). One of the water supply wells (35 to 40 feet deep) was located in the center of an area served by septic systems and within 100 feet of a landfill. During dry periods, heavy pumping of the well is postulated to have created a substantial cone of depression around the well, increasing the gradient and, thus, the flow rate from the surrounding septic systems (Wellings et al. 1977). Water from these 35- to 40-foot deep wells was pumped, chlorinated a short distance from the wells, and discharged into the distribution system.

Ten 100-gallon samples of potable, chlorinated water were tested for bacterial and viral occurrence. Isolation of an echovirus 22/23 complex in the 100-gallon samples was reported. The echovirus was isolated from treated well water containing 0.4–0.6 milligrams per liter (mg/L) residual chlorine. The virus was probably present as the result of contamination from septic systems and existed in chlorinated water in the absence of evidence of bacterial contamination. The chlorine appeared to have eradicated the bacteria. Fifteen cases of fulminant hepatitis A occurred in the camp 6 weeks after the sampling during the suspected contamination event. The level of chlorination in the water was probably sufficient to produce bacteria-free water but could not inactivate the virus, which was probably solids-associated.

**3. Shigellosis, Richmond Heights, Florida**—Between January and March 1974, approximately 1,200 cases of gastroenteritis occurred in Richmond Heights, Florida, a community of 6,500 people. At least 10 culture-proven cases of shigellosis were identified (Weissman et al. 1976). A chlorinator pump provided inadequate chlorination and contributed to the disease outbreak. TC levels were elevated in at



least one well. A tracer dye added to a nursery school's septic tank located 38 meters from that well was identified in well water after a travel time of 9 hours.

**4. Gastroenteritis, Restaurant in Michigan**—Patrons of a restaurant in southeastern Michigan complained to health authorities that in January 1970, within 30 hours of a meal at a particular restaurant, they had suddenly become ill, with nausea, vomiting, and diarrhea (Mack et al. 1972). Testing showed that food samples did not contain bacteria associated with foodborne infection. This prompted testing of the restaurant's drinking water supply. Five-gallon samples were taken from the 30.5 meter deep well and concentrated by centrifugation. Poliovirus type 2 was isolated from the well supplying water to the restaurant. Coliform levels in the well ranged from 0–16/100 mL, but no *Salmonella* or *Shigella* were found. The source of contamination was a waste drain field that allowed sewage to enter the well by passing through clay, shale, and limestone. The well was located 91.5 meters from the edge of the wastewater drain field. Both vertical soil penetration and significant lateral viral movement through the aquifer were indicated. A chlorinator was subsequently installed to mitigate against future outbreaks.

**5. Gastroenteritis, South Dakota Campground**—A viral gastroenteritis outbreak, caused by the Norwalk virus, occurred at a South Dakota campground in 1986. This outbreak was attributed to a septic system situated uphill from a drinking water well used by the campground (CDC 1988). Dye injected into the septic tank confirmed that the well was contaminated by sewage. The well chlorinator was not continuously operating.

**6. Gastroenteritis, Yukon, Canada Restaurant**—In 1995, at least 18 restaurant employees and 108 transient patrons became ill (Beller et al. 1997). SRSV, *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* were identified in stool samples. Dye was visually and chemically detected in one well about 24 hours after being flushed into the septic pit located about 15 meters from the well.

**7. Hepatitis A, Spring in Buttermilk Falls, Kentucky**—In 1982, 69 cases of hepatitis A were associated with untreated spring water (Bergeissen et al. 1985). Dye flushed into a septic tank was detected in the spring 2 weeks later. The spring is located in Meade County, a county with a karst limestone hydrogeologic setting.

**8. Gastroenteritis, Resort on Drummond Island, Michigan**—In 1991, 30 cases of gastroenteritis occurred in a resort. Dye injected into the septic tank appeared in the well water in 2 days in a fractured bedrock hydrogeologic setting (Institute of Water Research 1992).

**9. Gastroenteritis, Camp Four Echoes in Coeur d'Alene, Idaho**—In 1996, 94 cases of gastroenteritis occurred at a Girl Scout camp. Dye injected into the septic tank did not appear in the well water, but the septic tank drainfield is located at an elevation 50-75 feet higher than the wellhead. The well is 110 feet deep and is located in fractured basalt (McMillan 1996). *Arcobacter butzleri* was identified in ground water at the site (Rice et al. 1999)

**10. Gastroenteritis, Big Horn Mountains, Sheridan County, Wyoming**—In February 2001, an outbreak of norovirus caused at least 35 people to become ill due to an overloaded septic system 90 feet away from a ground water system for a hunting lodge (Anderson et al. 2003). The lodge had recently been renovated and expanded, but septic system capacity was not increased to account for the increased load. Norovirus was detected in well water by reverse transcriptase polymerase chain reaction (RT-PCR). Three different types of norovirus DNA were detected in stool samples; DNA fragments from one matched those in the water sample. The fact that multiple strains of norovirus were detected in stool samples may be due to the fact that the outbreak stretched over several weeks, and the well may have been recontaminated more than once by new guests.

**11. Gastroenteritis, Central Wyoming Restaurant-** In 2001, 84 patrons of a restaurant became ill from drinking water at the restaurant. Three stool samples tested positive for norovirus using RT-PCR. The well water also tested positive for noroviruses. The well drew water from a fractured bedrock aquifer. (Parshionikar et al. 2003).

**12. Gastroenteritis, South Bass Island, Ohio-** South Bass Island on Lake Erie in Ohio experienced a waterborne disease outbreak caused by wide-scale contamination of ground water with septage. Between July 23 and September 12, 2004, 1,450 cases of gastrointestinal illness were reported. Etiological causes included *Campylobacter*-type bacteria (16 cases), norovirus (9 cases), *Giardia* (3 cases), and *Salmonella* (1 case) (Ohio Department of Health 2005).

The island's winter population is only 500, but as many as 25,000 people per day visit the island in the summer (Ohio EPA 2005). Widespread use of onsite wastewater septic systems despite unsuitable karst geology may have contributed to the contamination (CDC 2005a). The island has 476 residential and many commercial onsite wastewater septic systems (CDC 2005a).

All 18 TNCWSs were positive for indicators at the time of the outbreak investigation. *Arcobacter butzleri* was detected in one well (CDC 2005a). Some TNCWSs were also positive for *Campylobacter*, *Salmonella*, adenovirus, and enterovirus DNA by PCR. However, PCR does not demonstrate that active infectious agents are present.

### 3.3 Wastewater Collection Systems

Wastewater collection systems consist of pipe lines that collect sewage and transfer it to a wastewater treatment facility. It is not uncommon for a community to locate sewer lines and water distribution lines in close proximity to each other, but preventive measures are typically effective in preventing cross-contamination. However, sewer lines are constructed from pipe segments that may leak at connection points or simply as a result of pipe deterioration with age. When leaky sewage pipes are in close proximity to PWS wells, source water contamination may result.

Wastewater contains fecal bacteria and enteric viruses. Wastewater differs from septage because the much greater numbers of people served by the sewer system make it more likely that a wider variety and perhaps higher density of pathogens (and indicator organisms) will be present in the wastewater. Melnick and Gerba (1980) compiled available data on the concentration of enteric viruses in untreated domestic sewage from Texas, Florida, Hawaii, Ohio, and California. They report average enteric virus concentrations of 50 to 250 PFU/L. Metcalf et al. (1995) report that raw sewage virus concentration can range from 100 to 1,000 PFU per 100 mL. Gerba et al. (1996) report rotavirus concentrations in raw sewage from the United States of 1 to 374 MPN per liter. Horstman et al. (1973) show that when only 0.4 percent of the population has been given the oral polio vaccine, that is sufficient to make that enteric virus detectable in the sewage emanating from that community.

Sorber (1983) reported raw sewage concentrations of 170 million colony forming units (CFU)/100 mL for TC, 44 million CFU/100 mL for fecal coliform, and 8.6 million PFU/L for bacteriophage. Geldreich (1996) reported microbial indicator densities in raw sewage from cities in the United States. The average values are: TC -  $2.19 \times 10^7$  per 100 mL (data from 14 cities); fecal coliform  $8.26 \times 10^6$  per 100 mL (data from 21 cities); and fecal streptococci-  $1.61 \times 10^6$  per 100 mL (data from six cities).

Pipes (1978) presented some estimates (based on dilution of feces with measured microbial concentrations) of bacterial indicator and pathogen density in wastewater collected from large populations. Assuming that only one person in 100,000 is infected with *Salmonella typhi*, then wastewater would be expected to have an *S. typhi* density of one organism per liter.

Disease outbreaks have been linked to wastewater collection system contamination of drinking water supply wells (Exhibit 3.2).

### Exhibit 3.2 PWS Waterborne Disease Outbreaks Associated with Wastewater Collection Systems

Outbreak Summary	Pathogen and Concentration in Well Water (If Identified)
Gastroenteritis and hepatitis A in Georgetown, TX	Coxsackie B virus (13 PFU/100 L)
Gastroenteritis in a country club in Hobbs, NM	Not available
Shigellosis in a resort and private homes in Island Park, ID	Not available
Gastroenteritis in New Braun, TX	Not available
Gastroenteritis in Crater Lake, OR	Enterotoxigenic <i>E. coli</i>

**1. Gastroenteritis and Hepatitis A, Georgetown, Texas**—In June 1980, a large outbreak of waterborne disease occurred in Georgetown, Texas (Hejkal et al. 1982). Georgetown is located about 20 kilometers (km) from the site of the 1998 *Cryptosporidium* outbreak in the Brushy Creek ground water PWS, Round Rock, Texas. Both are located in the Edwards Plateau karst aquifer. The outbreak was characterized by acute onset of diarrhea, abdominal cramps, nausea, and fever. About 79 percent of the total population of 10,000 people were thought to have had symptoms after consuming contaminated ground water. The community also had an excess of hepatitis A; 36 cases were reported in July, compared with an expected incidence of zero to two cases per month.

At the time of the outbreak, drinking water was chlorinated and the system pumped water to a common reservoir (Hejkal et al. 1982). Numerous causative agents were cited, including chlorination inadequate to treat sudden additional contaminant loads (i.e., insufficient monitoring to detect contaminant load changes), an extremely porous karst limestone aquifer supplying the water, and septic systems in the vicinity of the aquifer recharge zone. Despite a total chlorine residual of 0.8 mg/L in at least some distribution system samples, human enteric viruses were isolated from the distribution system.

An epidemiological survey indicated that the Georgetown outbreak was associated with water from central-city wells. The four central-city wells that serve the area range from 57 to 64 meters deep. Chlorine was injected as the water went to an underground storage tank at the time of the outbreak. Chlorination may have been inadequate to handle the increased contaminant loads, particularly if a sewage line leak occurred that made its way to a PWS well. The large number of illnesses suggest a dense contamination source, such as a leaky sewage line. Sewer lines within 15 m of the wells were tested with smoke and dye and no leaks were found. No other contamination source was identified.

Samples of sewage, well water, and tap water were taken on June 19, 6 days after the peak of the outbreak. Sewage samples contained enterovirus, rotavirus, and hepatitis A virus antigen (an indirect test for the presence of hepatitis A virus). Enterovirus was found in five sewage samples ranging from 1,200 to 7,400 PFU/100 L (an average of 4,580 PFU/100 L). Of the 42 plaques that were obtained from the cell

inoculation, one was coxsackie virus B2, and 19 were coxsackievirus B3. Rotavirus was detected in two sewage samples. HAV antigen was detected in three of five sewage concentrates.

Enteroviruses were found in two of the wells at 3 and 13 PFU/100 L, respectively. Of 25 plaques harvested, six were coxsackievirus B2, and one was coxsackievirus B3. Rotavirus was not detected in the well water. HAV antigen was detected in one well sample.

TCs and fecal streptococci were too numerous to count from one of the wells sampled on June 19. Bacteria were present from June 25 through July 27 in four central-city wells, as well as an additional well. Specifically, larger numbers of fecal coliforms were present in the central-city wells on June 26, a week after the second peak of gastroenteritis. Even after wells were treated with sodium hypochlorite on June 30, samples showed fecal coliforms in two wells. All wells tested negative for fecal coliforms after July 8. Of 125 bacterial samples taken from the distribution system from June 16 to July 23, none were positive for coliform organisms.

**2. Gastroenteritis, Country Club in Hobbs, New Mexico**—In July 1997, 123 country club patrons and employees became ill. The water was not routinely chlorinated, although the well water was shock chlorinated in 1995 after repeated positive coliform samples. Sewage line leaks had been reported and repaired in 1995. Stool samples yielded SRSV (now norovirus) and *E. coli* 086a:H11, a strain not previously associated with gastrointestinal or other disease (unpublished report, New Mexico Department of Health, Office of Epidemiology 1998).

**3. Shigellosis, Resort and Private Homes in Island Park, Idaho**—In August 1995, 82 cases of gastroenteritis were identified (CDC 1996). Fifteen shigellosis cases were confirmed at the resort, and an additional six cases were identified in nearby private homes. Illness at the resort was associated with tap water consumption. Sewage was draining improperly in the sewer lines, but no breaks were identified.

**4. Gastroenteritis, New Braun, Texas**—In May 1984, 251 people became ill with gastroenteritis. Blood serum antibodies showed that four of six ill people were exposed to Norwalk virus. The well water was chlorinated. Dye introduced into the sewage system appeared in the well water, but the exact site of sewage leakage or water contamination was not identified (D'Antonio et al. 1985).

**5. Gastroenteritis, Crater Lake, Oregon**—In 1975, 2,200 people became ill from *E. coli* O6:K15:H16 due to sewage overflow into the source water for two chlorinated springs. Fluorescent dye tracer in the sewage was recovered in the drinking water (Rosenberg et al 1977)

### **3.4 Wastewater Effluent and Biosolids Disposal**

Land application of wastewater effluent and biosolids from sewage treatment plants and septic tank residual material (sludge) is a common disposal/reuse method; if done improperly, this use may be another source of microbial ground water contamination. Sewage biosolids (sludge) are the residue generated during treatment of domestic sewage and are used as a soil conditioner and partial fertilizer. EPA is the primary federal agency responsible for sewage sludge management and encourages the beneficial use of this biosolid material. Land application is defined by regulation as the spraying or spreading of sewage sludge onto the land surface; the injection of sewage sludge below the land surface; or the incorporation of sewage sludge into the soil so that sewage sludge can either condition the soil or fertilize crops or vegetation grown in the soil (§503.11 (h)) (required by Section 405(d) of the Clean Water Act of 1977).

Pathogens present in raw sewage sludge include *Salmonella*, *Shigella*, *E. coli*, rotavirus, hepatitis A virus, adenoviruses, and enteroviruses (Akin et al. 1978). In one investigation, viruses were recovered from soils beneath sludge lagoons, but were not isolated in ground water monitoring wells adjacent to the site (Vaughn and Landry 1983). In another study at a wastewater effluent disposal site in Long Island, New York, with ground waters less than 35 feet deep, viable enteroviruses were recovered from the ground water (Vaughn et al. 1978). Both investigations were undertaken in sandy soils and aquifers.

One disease outbreak has been clearly linked to wastewater effluent or biosolids disposal contamination of drinking water supply wells (Exhibit 3.3).

### Exhibit 3.3 PWS Waterborne Disease Outbreaks Associated with Wastewater Effluent or Biosolids Disposal

Outbreak Summary	Pathogen and Concentration in Well Water (If Identified)
Gastroenteritis at a resort in northern Arizona	Not available

**Gastroenteritis, Resort in Northern Arizona**—In April and May 1989, 110 people became ill (Lawson et al. 1991). Serum antibodies for seven patients indicated illness from Norwalk virus. At the time of the outbreak, only three of five sewage treatment plant effluent leach fields were functional. The three functioning leach fields were carrying more effluent than they were designed to carry. Dye tracing from the leach field reached the wells in 3 to 11 days. The chlorinator for the well was also not working. The site was located in a fractured bedrock region.

### 3.5 Stormwater Infiltration

A study by Rose et al. (2000) evaluated waterborne disease outbreak data from 1971 through 1994 for ground water and surface water in 2,105 U.S. watersheds. Between 20 and 40 percent of the outbreaks were associated with extreme precipitation. This relationship was statistically significant for both surface water and ground water, although it was more apparent with surface water outbreaks. Therefore, stormwater run-off associated with extreme precipitation may in some cases be directly linked to waterborne disease outbreaks.

Urban and rural stormwater run-off can pick up pathogens from surface sources and enter ground water through a variety of pathways. Urban stormwater run-off has been found to have measurable concentrations of bacteria including: *Salmonella* at concentrations as high as 10/100 mL, *Shigella*, *E. coli*, and *Pseudomonas* (an opportunistic pathogen) (Pitt et al. 1994). Geldreich (1996) reported opportunistic bacterial concentrations in Baltimore stormwater of 1,000 to 100,000 per mL for *Pseudomonas aeruginosa* and 10 to 1,000 per mL for *Staphylococcus aureus*. *Salmonella* and enterovirus densities were lower, ranging from 10 to 10,000 per 10 liters of stormwater. Geldreich (1996) also reported microbial indicator data from stormwater-only sewers in Ann Arbor, Michigan, and a CSO from Detroit. As would be expected, the TC density was about one-log greater and the fecal coliform density was about two-log greater in the CSO water. Fecal streptococci density in CSO water was greater, although still on the same order of magnitude as stormwater.

Viruses have been detected in ground water where stormwater recharge basins were located short distances above the aquifer (Pitt et al. 1994). Urban run-off may come into contact with pet feces,

improperly disposed solid wastes (e.g., soiled disposable diapers), confined animal areas (e.g., kennels and veterinary clinics), and failing septic system effluent that is exposed on the ground surface. Infiltrating stormwater is of lower ionic strength (dissolved species concentration) than wastewater (Bitton and Gerba 1984), and this variable chemistry may change the attraction forces that govern the attachments between small particles. Thus, in the subsurface, viruses and bacteria may be detached from the particles to which they have attached and migrate into or with the ground water.

In urban settings, stormwater is often collected and allowed to infiltrate to ground water via settling basins. Pitt et al. (1994) estimated that the ground water contamination potential from enteroviruses due to stormwater infiltration to ground water is high compared to the contamination potential from *Shigella*, *Pseudomonas aeruginosa*, and protozoa, because viruses are among the most mobile pathogens in the subsurface environment. Municipalities may also collect stormwater and directly inject it into the ground via injection wells. Again, the potential for directly injected stormwater to contaminate ground water is expected to be higher for viruses (Pitt et al. 1994). Medium-density residential and commercial urban areas were found to contribute greater numbers of bacteria to stormwater than were low-density residential and undeveloped areas (Pitt et al. 1994). Contaminated stormwater may also enter ground water via improperly constructed or maintained wells, as well as improperly abandoned wells. Reneau et al. (1975) reported contamination of surface waters with fecal bacteria following precipitation events in which septic tank effluent was flushed by run-off into the surface waters. These contaminated surface waters may subsequently flood a wellhead and contaminate a well.

One disease outbreak has been clearly linked to storm water infiltration into a PWS well (Exhibit 3.4).

#### **Exhibit 3.4 PWS Waterborne Disease Outbreaks Associated with Storm Water Infiltration**

<b>Outbreak Summary</b>	<b>Pathogen and Concentration in Well Water (If Identified)</b>
Gastroenteritis in Philadelphia, PA	Not available

**Gastroenteritis, Ice Manufacturer in Philadelphia, Pennsylvania**—In 1987, after attending a football game in Pennsylvania, 84 students had symptoms of gastrointestinal illness, including nausea, vomiting, diarrhea, headache, and other symptoms (Cannon et al. 1991; CDC 1987). Approximately 92 percent of the affected students had purchased soda with ice from the stadium concessionaire. Later in the same month, 55 football team members became ill. At the same time, another outbreak resulting from consuming ice occurred in Wilmington, Delaware, in which 53 people were ill. Blood serum studies indicated that at least 13 people had exposure to Norwalk virus (now norovirus) and a virus particle similar to Norwalk was found by electron microscopy in one stool sample.

The ice served in the drinks at both sites came from the same supplier. The ice was traced to a manufacturer whose well and septic tank had been flooded by the Conestoga Creek after a torrential rainfall. The well water was not chlorinated during or after the flooding. High concentrations of fecal coliform bacteria were found in the ice and well water but no pathogens were detected. As a matter of policy, when untreated ground water used to make ice is involved in an outbreak and no source of fecal contamination is identified, the Centers for Disease Control (CDC) assume the ice outbreak is foodborne

rather than waterborne, so this outbreak was not included in CDC's surveillance reports on waterborne disease outbreaks.

### **3.6 Municipal Solid Waste**

Municipal solid waste includes discarded household and commercial products and wastes that can contribute pathogens to the solid waste material. Household wastes that contribute large numbers of microorganisms include: facial tissues, pet feces carrying human viruses, soiled disposable diapers, and decaying foods (Pahren 1987). Commercial solid wastes, such as food waste and food processing waste, may contribute pathogenic organisms to municipal waste landfills. Based on one test, the ratio of fecal coliform to fecal streptococci found in landfill waste suggests that pathogens in municipal waste are predominantly of non-human, warm-blooded animal origin (human feces has a much higher fecal coliform density than most animals) (Pahren 1987).

As waste accumulates at landfills, rainfall percolates through the waste and generates leachate, generally high in dissolved constituents and capable of supporting pathogenic bacteria. Municipal solid waste and undigested sewage sludge have comparable concentrations of total and fecal coliform bacteria (Pahren 1987). Large numbers of microbes have been found in both solid waste and the leachate collected from beneath solid waste facilities (Pahren 1987). Geldreich (1996) reports that sanitary landfill leachate examined 70 days after waste emplacement had fecal coliform density of 33,000 per 100 mL and fecal streptococci density of 170,000 per 100 mL. Cooper et al. (1975) examined leachate from domestic solid waste in simulated landfills and found that polioviruses were sporadically recovered for up to 20 weeks, indicating that the leachate was not toxic to the poliovirus.

The Code of Federal Regulations (40 CFR 258) provides regulations for municipal solid waste landfills to prevent fecal contamination of ground water. These regulations provide siting restrictions, operating and design criteria, and ground water monitoring requirements to minimize the possibility of fecal contamination (and other contamination). These regulations apply to larger landfills; smaller ones are not similarly regulated. In some areas of the country, smaller landfills may not be located at a sufficient distance above the water table to prevent contamination of ground water. If landfills are not properly lined or do not have leachate collection and treatment systems, they may serve as pathways for microbial movement into the ground water.

At this time, no PWS waterborne disease outbreaks have been reported as resulting from contamination by a municipal solid waste contamination source.

### **3.7 Animal Sources of Contamination**

Microbial diseases in humans may originate in animal sources. Animals that carry bacteria that are pathogenic to humans include beavers, migratory birds, muskrats and other rodents, and livestock (Hurst and Murphy 1996). Cattle, particularly dairy cows, are an important reservoir of *E. coli*, although serotypes pathogenic to humans have been reported to be present in less than 9 percent of farm animals in the United States (Geldreich et al. 1992). Geldreich (1996) reported that fecal pollution loads in stormwater run-off from one feedlot were similar to the load in the raw sewage discharge from a city of approximately 10,000 people. Bacterial contamination can transfer from one species to another; dairy cows have been infected with *Salmonella* after ingesting hay covered in contaminated bird droppings (Glickman et al. 1981).

The available data suggest that only a very limited number of enteric viruses pathogenic to humans have an animal reservoir (USEPA 1999a). Rotavirus, a cause of gastroenteritis, may cross-infect humans and a variety of animals including cattle (Hurst and Murphy 1996). Astrovirus has been isolated from many animals, including calves, lamb, pigs, cats, dogs, turkeys, and ducks (Kurtz and Lee 1987). These astrovirus strains may be transmitted in water, although infection may or may not be associated with illness (Kurtz and Lee 1997). In 1994, it was reported that a single individual was infected by a bovine rotavirus (Nakagomi et al. 1994). Most recently, Schlauder (1998) found that a hepatitis E virus (HEV) strain from U.S. swine was genetically very similar to a hepatitis A strain isolated from a U.S. patient and HEV antibodies were prevalent in U.S. rodents, suggesting a possible swine or rodent animal reservoir for the agent (Smith 2000, Favorov et al. 2000)

Where animal wastes are applied to the land intentionally or unintentionally, ground water may become contaminated. Accumulation of animal manure in unlined lagoons or directly on the surface of the ground can serve as a source of fecal ground water contamination. Slurried manure from dairy cows supported viable *Salmonella* populations for up to 286 days (Glickman et al. 1981), providing sufficient viability for these bacteria to reach shallow ground water from an unlined lagoon. Injection of animal waste into the soil, over-application on crops, disposal directly onto the ground surface, and accumulation of manure slurries in wastewater lagoons can contribute to the ground water contamination in a similar manner to that of land application of human waste.

In agricultural areas, manure spreading practices can contribute to fecal contamination of ground water following precipitation events (Pitt et al. 1994). Crop lands fertilized with animal waste and underlain with perforated pipe to enhance drainage may be another source of fecal contamination of ground water. Drain tiles were identified as a pathway contributing to fecal coliform contamination of ground water 2 to 4 orders of magnitude greater than in non-tiled areas (Reneau et al. 1975). Stormwater run-off from agricultural lands in rural South Carolina was identified as one of the sources of microbial contamination of ground water (Sandhu et al. 1979).

EPA, along with the U.S. Department of Agriculture, has developed the Unified Strategy for Animal Feeding Operations to minimize the adverse effects of nutrients, organic matter, and other contaminants on water quality. While not specifically targeted toward fecal contamination of ground water, the strategy should have the ancillary effect of minimizing animal sources of fecal contamination from AFOs and CAFOs. Under the strategy, all AFO owners and operators should develop and implement nutrient management plans with actions dealing with manure handling and storage that should minimize nutrient and accompanying fecal contamination of water, including ground water.

Exhibit 3.5 lists two outbreaks that resulted from contamination of ground with animal waste, one from wildlife and the other from livestock.



### Exhibit 3.5 PWS Waterborne Disease Outbreak Associated with Animal Waste

Outbreak Summary	Pathogen and Concentration in Well Water (If Identified)
Gastroenteritis, bloody diarrhea and kidney failure (hemolytic uremic syndrome) in Alpine, WY	<i>Enterococcus faecium</i>
Gastroenteritis and kidney failure (hemolytic uremic syndrome) in Walkerton, Ontario	<i>E. coli</i> (20 CFU/100mL) (subsequently some or all identified as <i>E. coli</i> O157:H7)

**1. Alpine, Wyoming**—A waterborne disease outbreak occurred in Alpine, Wyoming in 1998 (Olsen et al. 2002). Of the 157 ill individuals, 71 had laboratory confirmed *E. coli* O157:H7 in stool samples (*Enterococcus faecium* was identified in the drinking water). Four persons (three children) had kidney failure (hemolytic uremic syndrome). The spring water supply system was contaminated by deer and elk feces that leached into the town's unconfined aquifer (Olsen et al. 2002).

**2. Walkerton, Ontario**—A large outbreak occurred in Walkerton, Ontario in 2000 (Health Canada 2000). The population of Walkerton is about 5,000 people, and the number of ill residents and visitors was approximately 1,350. Sixty-five people were hospitalized, 27 developed kidney failure, and 6 people died. One hundred and sixteen people had stool-confirmed cases of *Campylobacter* infection. *E. coli* O157:H7 was found in the well water. Cattle wastes were likely flushed from nearby farms directly into the well or through fractures or solution-enhanced (karst) features in the aquifer.

In addition to the outbreaks at PWSs discussed above, a notable large outbreak occurred in a ground water system not meeting the criteria to be classified as a PWS. In 1999, an outbreak of gastroenteritis and kidney failure (hemolytic uremic syndrome) occurred at a fair in Washington County, New York (Centers for Disease Control and Prevention 1999, New York State Department of Health 2000). This outbreak was associated with cattle wastes containing *E. coli* O157:H7. Cattle wastes from a nearby barn entered the well directly, or indirectly via infiltration through the sandy soil and aquifer or were transported on muddy boots into the bathroom floor drain and entered the ground water via a nearby septic system. In this outbreak, there were 781 illnesses, 71 hospitalizations, 14 cases of kidney failure, and 2 deaths. One well tested positive for *E. coli* O157:H7. Forty-five illnesses were associated with *Campylobacter jejuni*. To date, this is the largest *E. coli* O157:H7 outbreak reported in the United States, from either food or water sources.

Other waterborne disease outbreaks from ground water sources have been linked to contamination by parasitic protozoa (i.e., *Giardia* or *Cryptosporidium*), which are commonly associated with animal sources. However, ground water sources with these types of contaminants are defined as ground water under the direct influence of surface water (GWUDI). As discussed in section 2.0, GWUDI systems are regulated under surface water treatment technique requirements.

### 3.8 Storage and Distribution System Contamination

The purpose of this section is to describe the microbial contamination sources and events that may affect PWS storage and distribution systems, especially those PWSs using ground water. Contamination within the distribution system may occur in PWSs using ground water even if the raw water poses no public health risk. Surface water systems may provide some measure of protection against such

contamination since they are required to maintain a disinfection residual at the entry point to the distribution system; ground water systems, however, are not subject to this requirement and are therefore more vulnerable if the distribution system is contaminated. Uncontaminated ground water supply systems may become contaminated if untreated water is stored in uncovered or improperly sealed tanks.

Disease outbreaks caused by *Salmonella typhimurium* and *Campylobacter jejuni* in Minnesota and Missouri in 1993 were attributed to contamination of stored water by feces from birds and small mammals (Kramer et al. 1996). Kramer et al. (1996) recommended exercising caution when cleaning storage towers to prevent flushing of stagnant water or sediment from the storage tank into the distribution system. Sanitary surveys are important protections against microbial contamination of storage and distribution systems. EPA recently issued sanitary survey guidance to facilitate such inspections in conjunction with promulgation of the Interim Enhanced Surface Water Treatment Rule (IESWTR) (USEPA 1999b) and is developing similar guidance tailored to ground water systems to accompany promulgation of the Ground Water Rule (GWR).

Inadequately disinfected distribution systems, including storage towers, can develop microbial biofilms on the inside walls of the pipes. Initially, biofilms may function as a filter, adsorbing pathogens (Seunghyun et al. 1997). Ultimately, biofilm material (and associated pathogens) may be shed (sloughed) from the pipe walls thereby contaminating the drinking water at the tap, depending on the presence of a disinfection residual in the distribution system.

Disease outbreaks have been linked to fecal contamination of ground water distribution systems in four cases (Exhibit 3.6).

### Exhibit 3.6: PWS Waterborne Disease Outbreaks Associated with Ground Water Storage or Distribution Systems

Outbreak Summary	Measured Pathogen Concentration in Drinking Water Well (If Identified)
Salmonellosis in Riverside, CA Salmonellosis in Gideon, MO Gastroenteritis in a resort in MN Gastroenteritis and hemolytic uremic syndrome in Cabool, MO	<i>Salmonella typhimurium</i> (17 MPN/L) <i>Salmonella typhimurium</i> (1 CFU/gallon) <i>Campylobacter jejuni</i> Not Available

**1. Riverside, California**—In May and early June 1965, an estimated 16,000 people became ill. At least 100 stool samples were positive for *Salmonella typhimurium*. No contamination source was identified. A composite water sample based on 74 samples collected from distribution systems (including home taps and storage reservoirs) had an average concentration of 17 MPN/L (Boring et al. 1971). No other contamination source was identified.

**2. Gideon, Missouri**—In December, 1993, an estimated 650 people became ill with gastroenteritis, and seven nursing home residents died. Sediment from a water storage tank was found to contain *Salmonella typhimurium* and a possible entrance passageway for birds was identified. Thus, the tank was identified as the likely source of contamination. One of six 1-gallon samples (taken from a fire hydrant) was found to be positive for *Salmonella typhimurium* (Angulo et al. 1997; Kramer et al. 1996).

**3. Resort in Minnesota**—In November 1993, *Campylobacter jejuni* caused 32 illnesses at a resort where fecal coliforms were found in a storage tower containing untreated well water. The storage tower had been cleaned the month before (Kramer et al. 1996).

**4. Cabool, Missouri**—In 1990, 243 people became ill with gastroenteritis (Swerdlow et al. 1992). Two people developed hemolytic uremic syndrome (kidney failure) and four nursing home residents died. Twenty-one people were identified as ill from *E. coli* O157:H7. An untreated ground water distribution system became contaminated but an actual contamination source was not identified. It is possible the system became contaminated after replacement of 45 water meters. Replacement of water mains occurred after an increase in *E. coli* cases was observed. (Swerdlow et al. 1992; Geldreich et al. 1992). This system was not chlorinated following maintenance. Seepage from sewer lines and sewage overflows were identified as the likely source of the fecal contamination (Swerdlow et al. 1992). It is possible that karst hydrogeology may also have played a role.

Rice et al. (1992) show that the survival rates (in ground water) of *E. coli* O157:H7 samples implicated in the Cabool outbreak were similar to an Ohio River strain of typical *E. coli* (i.e., a strain that does not cause illness). At 5 degrees C, the die-off was about 3.5 log<sub>10</sub> reduction after a 70-day incubation period.

### 3.9 Conclusions

There are many fecal contaminant sources, including both surface (e.g., CAFOs, wastewater lagoons, etc.) and subsurface (e.g., improperly designed, sited, operated, or maintained septic systems) sources. These contaminant sources can infiltrate ground water drinking water supplies, which, if inadequately treated, can lead to waterborne disease outbreaks. The case studies summarized in this chapter show that contamination outbreaks can result from many different scenarios. The potential for future drinking water disease outbreaks can be minimized through preventive measures such as those prescribed by the GWR.



## 4.0 Microbial Contaminant Fate and Transport

### 4.1 Background

The fate and transport of bacteria and viruses in the subsurface environment are major issues with respect to human exposure to waterborne pathogens. The ability of microorganisms to survive in the environment allows them to be transported by water, food, or personal contact to a human host. The Ground Water Rule (GWR) is concerned with transportation of contaminants to ground water sources from which drinking water is drawn. Pathways such as flowing ground water that supplies public water system (PWS) wells via infiltrating recent surface water or improperly constructed wells are of particular concern.

Contamination can reach ground water directly by transport through soil openings and through joints, fractures, or fissures in rock. Also, direct transport is more likely in areas where soils are highly permeable. Thin or absent soils are typical on steep slopes in mountainous regions, in regions where the soils were removed by glaciers during the last Ice Age, or in areas where poor soil conservation practices have resulted in significant soil erosion.

All microbial contaminant sources that enter ground water either where soils are absent or through the wellhead of an improperly constructed or abandoned well bypass an important protective barrier. The soil zone protects by providing in situ treatment that minimizes public health risks. However, the capacity of a soil to attenuate contamination depends on soil types, soil saturation, and source of contamination. Thus, the presence of soil does not guarantee that a barrier to contamination exists.

Several examples provide insight into pathways by which contaminants can enter ground water. In the example of an improperly sited septic system located in an area with a high water table, the microbial contamination is introduced directly into an aquifer, thereby bypassing the protective attenuation action of the unsaturated soil. The septic system's drainfield lines discharge leachate directly into the aquifer. A more direct pathway through which contaminants can reach an aquifer is in stormwater from an overflowing sewage lagoon or from combined sewer overflow (CSO) releases. Stormwater, for example, may percolate downward directly into the subsurface. However, most stormwater runs off into surface water and may then enter ground water through surface water infiltration or recharge. A special case of recharging to ground water is induced infiltration of surface water by the action of pumping wells. Under certain hydrogeologic and hydrologic conditions, rivers, lakes, and reservoirs can act as recharge sources for underlying aquifers. Therefore, these bodies of water also can act as pathways to an aquifer for any contaminants present in stormwater or surface water. Whatever the pathway, the stormwater or contaminated water can be diluted by surface water and then flow through the aquifer to a well.

Sections 4.2 and 4.3 describe the factors governing viral and bacterial fate and transport, respectively. Section 4.4 discusses characteristics of wellhead protection and aquifer exploitation near ground water intake points. Section 4.5 discusses conditions at and near wellheads that may allow microbial contaminants to bypass the naturally protective features of the subsurface and take a more direct path from the surface to the well intake point. The last section in this chapter, section 4.6, describes

the public health risk associated with microbial pathogen transport through the subsurface to a PWS well, with emphasis on waterborne disease outbreaks attributable to this method of transportation.

## **4.2 Factors Affecting the Fate and Transport of Viruses in the Subsurface**

The role of hydrology is paramount when determining the transport characteristics of a plume of contaminants. This section describes the hydrogeologic features that govern virus fate and transport in the subsurface. Virus fate and transport in the subsurface are influenced by numerous factors (adapted from Yates and Yates 1988 and Mattle et al. 2001), including:

- Temperature
- Hydrogeologic conditions
- Soil properties, including mineral coatings on grains
- Water pH
- Conductance of aquifer material
- Inorganic ions/salt species and concentration
- Organic matter
- Virus type and degree of aggregation
- Microbial activity
- Iron content
- Moisture content

The transport and persistence of a virus in the subsurface, that is, the unsaturated zone (typically consisting of the soil at the top of the uppermost aquifer) and the saturated zone (typically consisting of one or more aquifers), are important factors in the occurrence of microbial contamination. Locally, weather changes may alter the hydrogeologic environment. For example, wetter (high precipitation) conditions may result in high water tables in an unconfined aquifer, thereby potentially reducing the distance and time required for viruses to enter the now shallower aquifer. Precipitation recharge and other hydrogeologic factors that govern the flow of ground water are much better documented than the factors that govern the transport of microbial contaminants within that ground water.

Factors affecting virus fate and transport are complex, interrelated, and poorly understood. In the laboratory, soil columns are typically used to study and observe virus transport under controlled conditions. The applicability of soil column studies to natural hydrogeologic conditions is uncertain. Typically, soil column studies are conducted over a short time period to minimize virus die-off during the experiment. Virus survival assessment is typically conducted in the laboratory using natural ground water to better represent actual water chemistry conditions. These survival experiments are designed to measure

virus survival in static ground water and do not typically consider the effects of virus interactions with solid material that might represent a soil or aquifer or the movement of water and viruses through the soil or aquifer material.

The following discussion of the parameters important to virus fate and transport discussion is based on studies using both viral pathogens, such as enteroviruses, and studies using pathogen indicator viruses, bacteriophage, and coliphage, a subset of the bacteriophage. Bacteriophage are viruses that infect bacteria only; coliphage are likely to primarily infect coliform bacteria. The data from both enteroviruses and the bacteriophage are combined in the discussion; it is assumed that physical properties such as soil moisture content, if important to the fate and transport of a bacteriophage, will also be important to the fate and transport of the pathogenic viruses. Because of the variability inherent in viral strains, this assumption may not always apply.

Exhibit 4.1 identifies the important factors governing microbial transport in ground water. The discussion about ground water flow is restricted to the elements considered to be most important for determining microbial hazard to a PWS well, but is not intended to be comprehensive. Additional information about ground water flow (under either saturated or unsaturated conditions) can be obtained from any hydrogeology textbook.

#### **Exhibit 4.1 Factors Influencing Virus Transport and Fate in the Subsurface**

<b>Factor</b>	<b>Influence On:</b>	
	<b>Fate</b>	<b>Transport</b>
Temperature	Viruses survive in soil and water longer at lower temperatures.	Unknown.
Hydrogeologic conditions and well pumping rate	A short ground water time of travel indicates that viruses may be transported to water supply wells before dying off or becoming inactivated.	Aquifer characteristics determine the type of ground water flow. Relatively slow flow reduces the rate of virus migration while conduit, fracture flow, or rapid flow in coarse-grained porous media enhances the transportation of viruses. Generally, virus migration increases with increasing hydraulic loads (pumping rates) and flow rates.
Soil properties; iron oxide coatings on soil or aquifer grains	Effects on survival are probably related to the degree of virus adsorption.	Greater virus migration in coarse-textured soils; there is a high degree of virus retention by the clay fraction of soil; iron coatings may be especially efficient in providing an attractive surface for virus attachment.
pH	Most enteric viruses are stable between a pH range of 3 to 9; survival may be prolonged at near-neutral pH values.	Generally, low pH favors virus adsorption and high pH results in virus desorption from soil particles.

Factor	Influence On:	
	Fate	Transport
Inorganic Ions / Salt species and concentration	Some viruses are protected from inactivation by certain cations; the converse is also true.	Generally, increasing the concentration of ionic salts and increasing cation valencies enhance virus adsorption.
Organic matter	Presence of organic matter may protect viruses from inactivation; others have found that it may reversibly retard virus infectivity.	Soluble organic matter competes with viruses for adsorption sites on soil particles.
Virus type	Different virus types vary in their susceptibility to inactivation by physical, chemical, and biological factors.	Virus adsorption to soils is probably related to physicochemical differences in virus capsid surfaces.
Microbial activity	Some viruses are inactivated more readily in the presence of certain microorganisms; however, adsorption to the surface of bacteria can be protective.	Unknown.
Iron content in shallow soil or aerobic aquifers	Unknown.	Iron oxidizing bacteria may form a biomass layer that filters out viruses. Heavy precipitation events may cause the ionic strength of the water to decline and the biofilms to release the filtered organisms.
Soil moisture content	Virus adsorption to particle surfaces and, hence, their survival, increases in unsaturated conditions.	Increased saturation promotes desorption of viruses from particle surfaces and migration in ground water.

Source: Adapted from Yates and Yates 1988.

**Temperature:** Temperature is the most significant element that governs virus survival, at least for the several enteroviruses and the coliphage MS-2 (Gerba et al. 1991). In general, viruses survive longer at lower temperatures. For the coliphage MS-2, the inactivation rate becomes very low at 8 degrees Centigrade (C) and the number of viruses decreases by an order of magnitude each month. At 15 degrees C, the number of viruses can decrease by as much as four orders of magnitude each month. Laboratory measurements of virus inactivation rates are available in the literature (Yates 1987), as well as mathematical expressions suitable for predicting virus inactivation as a function of ground water temperature (Yates 1987; Gerba, Yates, and Yates 1991). Average ground water temperatures at about 30 meters (m) below the water table in the continental United States vary from 4 to 25 degrees C.

Nasser and Oman (1999) studied temperature effects on virus inactivation in ground water, wastewater, and buffered saline. Male-specific bacteriophages (see Chapter 2) persisted where *E. coli* were inactivated in ground water at 4 and 37 degrees C. Therefore, male-specific bacteriophages were found to be more suitable for indicating the presence of viruses in ground water than *E. coli*, especially at lower temperatures.



In a recent compilation of virus inactivation rates in ground water, Hurst et al. (1997) attempted to apply an algorithm, developed for virus inactivation in surface water, to virus inactivation in ground water. The virus inactivation rates for surface water were found to differ substantially from the virus inactivation rates for ground water (in the same water temperature ranges).

**Hydrogeologic Conditions:** The migration and fate of viruses in ground water are strongly influenced by the hydrogeologic characteristics of aquifers and their overlying materials. In particular, the types of water-bearing openings in aquifers are critical factors in determining ground water flow and, hence, virus migration via ground water.

Surface and subsurface earth materials are highly variable in their degree of particle consolidation, the size of particles, the size and shape of pore or open spaces between particles and between cracks in consolidated rocks, and in the mineral and chemical composition of the particles. Ground water occurs both in loosely aggregated and unconsolidated materials, such as sand and gravel, and in consolidated rocks, such as sandstone, limestone, granite, and basalt (USGS 2001b).

Aquifers with a slow flow rate allow microbial contaminants more contact with aquifer materials and, thus, more opportunities to be adsorbed (attached to particulate matter) and/or filtered out of the ground water. When ground water flow rates are high, there are fewer opportunities for viruses to come in contact with the aquifer solid materials. As a result, the viruses tend to stay in the water rather than adsorb onto surfaces and, thus, are more readily transported through the aquifer.

The velocities of ground water flow are generally low and are orders of magnitude less than the velocity of streamflow. For comparison, stream flow is usually measured in feet per second, where one foot per second is equal to about 16 miles per day. The movement of ground water normally occurs as slow seepage through the pore spaces between particles of unconsolidated materials or through networks of fractures and solution openings in consolidated rocks. One foot per day or more is considered a high flow rate for ground water; flow rates can be as low as one foot per year or one foot per decade (USGS 2001b).

In karst aquifers, formed from limestones, as well as from some dolomites and marbles, fractures may be enhanced by solution weathering to form large cave systems that allow rapid and high volume ground water flow. This type of flow, known as conduit flow, reduces the opportunities available for viruses to adsorb to the conduit wall surfaces; thus, viruses are more readily transported through the aquifer.

Finally, the direction and distance that contaminants such as viruses can travel through aquifers under natural conditions are correlated to the movement of ground water along flow paths from areas of recharge to areas of discharge at springs, along streams, lakes and wetlands, or when pumped at wells. The length of the flow path for ground water within an aquifer ranges from a few feet to tens, and sometimes hundreds of miles. A deep ground water flow system with long flow paths between areas of recharge and discharge may be connected to one or more local flow systems. Therefore, defining a flow system is subjective and results obtained from studies depend, in part, on the scale of the study.

**Soil Properties:** Soils vary in their chemical and physical properties and these variations influence the fate of viruses. Soil characteristics depend on, among other things, the type of rock from which the soil is derived. Viruses are retained by soils through adsorption phenomena. Relatively coarse-grained materials, such as sandy soils, are poor adsorbers, while fine-grained soils, such as clay

soils and colloidal organic material, are good adsorbers (Keswick and Gerba 1980). The small sizes of these clay and colloidal particles present an overall large surface area per volume and, hence, a high number of potential adsorption sites for microbial contaminants. The clay mineral fraction of a soil readily adsorbs viruses due to its high number of potential adsorption sites (Gerba and Bitton 1984; Sproul 1973).

The attachment or adsorption of a virus particle to a solid surface may protect it from hostile environments (Vaughn and Landry 1983; Hurst et al. 1980) where natural chemical and biological degradation may occur. As a result, clay soils slow virus migration, but enhance survival (Bitton and Gerba 1984; Keswick and Gerba 1980). However, more recently, Pieper et al. (1997) and Ryan et al. (2002) found evidence that virus attachment may promote virus inactivation.

Adsorption is not permanent. The ionic strength or conductivity of liquids percolating past the adsorption sites plays an influential role in virus sorption and desorption (Vaughn and Landry 1983; Bitton and Gerba 1984; Yates et al. 1987; Mattle et al. 2001). Vaughn and Landry (1983) suggest that a reduction in ionic strength weakens the virus-soil adsorption forces and allows the virus to move more readily through the soil with the percolating fluid. Natural rainfall, with its extremely low ionic strength, may act to desorb and remobilize viruses that have adsorbed to the upper layers of the soil. If the soil becomes saturated, as during a heavy rainfall, the desorbed viruses tend to migrate to lower soil depths (Bitton and Gerba 1984; Vaughn and Landry 1983). The ability of rainwater to release viruses depends on the soil type and is more pronounced in sandy soils than in clay soils (Gerba and Bitton 1984).

The mineral coatings on aquifer grains, and, in particular, iron oxide coatings on quartz sand grains in a sand or sand and gravel aquifer, may be especially efficient at adsorbing viruses that are entrained in flowing ground water. Other mineral coatings may be more or less efficient at adsorbing viruses. A recent review article (Ryan and Elimelech 1996) addresses this issue in more detail.

**pH:** pH indirectly affects virus survival by controlling adsorption to soil particles. At neutral pH (pH=7), most enteric viruses are negatively charged (Yates and Yates 1988). At the same pH, most soils are also negatively charged, and viruses will attach to soil particles only with great difficulty (due to other factors dominating). As the pH of the soil changes, due perhaps to the effect of infiltrating, lower pH precipitation, the virus will become less negatively charged, and viruses may thus be more likely to attach to the soil particles.

Drewery and Eliassen (1968) showed that decreasing the pH of the soil reduces its negative charge and decreases the repulsion between the virus and soil. Scandura and Sobsey (1997) report an opposite effect— greater virus recovery in ground water at elevated ground water pH. Yates and Yates (1988) provide a more extensive literature review of pH effects. Redman et al. (1999) report that, for one particular virus (male-specific coliphage) studied, ground water pH was not a factor governing transport of the virus. Rather, the virus appeared to be unaffected by pH at the pH typical of ground water at the investigation site, and, thus, may be exceptionally mobile in ground water at that site because virus attachment (to particles and aquifer materials) is inhibited. At the same site, Yanko et al. (1999) reported the presence of both male-specific and somatic coliphage in 18 PWS wells within 500 feet downgradient of a reclaimed water and stormwater infiltration basin. It is possible that the one type of male specific coliphage studied may have migrated as much as 500 feet to the wells because it is insensitive to pH changes.

***Inorganic Ions:*** The types and concentrations of salts have a profound influence on the extent of virus transport to and within the subsurface. Transport slows in the presence of increasing concentrations of ionic salts and increased cation valencies due to increased virus adsorption. Conversely, a decrease in the salt concentration or ionic strength of the soil water can cause desorption of viruses from soil particles with readsorption occurring at greater depths (Yates and Yates 1988). Redman et al. (1999) report that, for the virus studied, small changes in the ground water hardness and total dissolved solids are significantly more important than ground water pH in explaining the transport of viruses. These results may explain the mobility of male-specific coliphage in the subsurface. Yanko et al. (1999) reported the presence of both male-specific and somatic coliphage in 18 PWS wells within 500 feet downgradient of a reclaimed water and storm water infiltration basin, as described in the paragraph above. Yates and Yates (1988) provide a comprehensive literature review of the effect of inorganic ions on virus survival and mobility.

***Organic Matter:*** The organic content of the soil also influences adsorption and survival of viruses. Humic and fulvic acids from leaves and other commonly occurring organic matter in the soils may cause loss of virus infectivity and prevent adsorption. Dissolved organic matter has generally been found to decrease virus adsorption by competing for adsorption sites on soil particles. If there are few adsorption sites in the soil or aquifer, then the virus will be more mobile in the subsurface because it will be less likely to become attached to soil or aquifer material. Pieper et al. (1997) and Ryan et al. (1999) examined the effects of organic matter entrained in a sewage plume on the mobility of viruses in that plume at a field site in Cape Cod, Massachusetts. Pieper found that the PRD-1 bacteriophage was about twice as mobile in the sewage plume as compared with above the plume probably because the organic matter occupied most of the available attachment sites within the plume. Ryan found that phosphate, as well as organic material, were occupying attachment sites, allowing enhanced PRD-1 mobility.

***Virus Type:*** Different viruses vary in characteristics such as mobility and their susceptibility to inactivation. Nasser and Oman (1999) and Hurst (1980) showed that the inactivation rates of seven different viruses varied, even when incubated under the same conditions. Regarding mobility, it has long been assumed that the electrical charge on the viral outer coat governed subsurface viral mobility, all other factors being equal. Most recently, Dowd et al. (1998) reported that, for the smaller bacteriophage, electrical charge was dominant. However, they found that the mobility of larger viruses such as PRD-1, was due more to size than electrical charge.

***Degree of Aggregation:*** The formation of viral aggregates may influence virus survival in natural waters (Yates and Yates 1988). Grant (1994, 1995) suggested a mathematical model for the survival of virus aggregates that accounts for the unexplained complexity inherent in simple models of virus survival.

***Microbial Activity:*** Biological inactivation is an important factor in virus survival. Viruses persist longer in sterile soils than in non-sterile soils (Vaughn and Landry 1983). Nasser et al. (2002) also showed that microbial activity enhanced inactivation in saturated soils, although the extent of inactivation depended on the virus type. However, there is no clear trend regarding the contribution of soil microflora to virus decline (Gerba and Bitton 1984).

***Iron:*** Viruses are strongly immobilized by iron oxides. The high affinity of iron oxides for viruses is complex and may be facilitated by iron-oxidizing bacteria (Bitton and Gerba 1984). Some species of iron-oxidizing bacteria secrete a polysaccharide mucus (gel) to maintain a distance from their iron oxide waste products. The polysaccharide gel/biomass mat may form a filter layer that prevents viruses from reaching the water that is withdrawn at well intake points. During high precipitation events

and the associated decline in ionic strength of the water, it is possible that the gel releases the filtered organisms. The released microbes may then enter water supply wells during pumping.

***Soil Moisture Content:*** The moisture content also influences movement of viruses through soil. Bagdasarjan (1964) reported a marked effect of moisture content on enteroviruses. Viruses survived no more than 15 to 25 days in air-dried soil compared with 60 to 90 days in samples with 10 percent moisture. DuBoise (1979) reported increasing reduction in poliovirus 1 in a dry, sandy soil during soil dehydration. Under unsaturated conditions, viruses get closer to particle surfaces, leading to increased opportunities for viruses to attach themselves to soil (Gerba and Bitton 1984). Virus survival is greater as the moisture content of the soil increases from an air-dried condition to a 10-percent moisture condition (Keswick and Gerba 1980). Viruses may become detached from soil particles and are therefore free to migrate to ground water as the soil moisture content increases to saturation levels. Most recently, Jin et al. (1998) summarized the literature and reported that the air-water interface is responsible for differing virus survival and transport in unsaturated, as opposed to saturated, soils. The coliphage  $\phi$ x174 and MS-2 were significantly more mobile in saturated as compared to unsaturated soils.

The rate at which water or effluents are applied to the soil affects the degree of virus removal or adsorption to the soil particles. The lower the rate of seepage into soil, the longer the retention of viruses within the unsaturated zone. This results in more efficient virus removal because of increased biological activity (Gerba and Bitton 1984). Yates and Yates (1988) have shown that the amount of virus removal increases as the application rate decreases.

### ***Transport Data***

There are a large number of important physical properties that govern virus fate and transport. When there are numerous opportunities for viruses to interact with the soil or aquifer media, interactions governed by these physical properties make it difficult to predict virus transport distances, even on a site-specific basis. In contrast, for hydrogeologic settings in which viruses are inhibited from interacting with the aquifer material, by, for example, large volume and rapid flow in a karst conduit, bedrock aquifer fracture or the large pores of a gravel aquifer (defined as sensitive aquifers), there are fewer opportunities for these complex interactions to have significance in governing virus fate and transport.

The available data for virus transport distances in various hydrogeologic settings illustrate the complexities of predicting virus transport in any physical medium, especially those in which there are significant interactions among the viruses, water and soil, or aquifer medium. Exhibit 4.2 shows migration distances for various virus types in soils and ground water based on an update of a compilation of data by Yates and Yates (1988).

Since the publication of Yates and Yates (1988) a large number of new virus transport studies have been conducted. These studies were typically virus tracer tests. A tracer test injects viruses directly into the aquifer, and samples are collected from downgradient monitoring and production wells to determine a virus transport distance (and arrival time). It should be recognized that these tests are typically minimum virus transport distances because the test is undertaken over a period of days to weeks, a period shorter than the survival time of viruses in the subsurface. Most studies are not designed to determine the maximum virus transport distance. Tracer tests are conducted under both natural gradient and forced gradient conditions. In a natural gradient test, there is no downgradient pumping well that increases the ground water flow potential. The new studies are summarized below and are included in Exhibit 4.2. Some new data (post-1988) in Exhibit 4.2 are shown in bold.

### Exhibit 4.2 Migration of Viruses in the Subsurface

Microorganism	Medium	Horizontal Distance (meters)
Viruses	Sand and coarse gravel	25
Bacteriophage	Sand Boulder clay Sandstone	400 510 570
F+RNA-phage	Sand	30 2 4
Coliphage f1	Sand and gravel	112.5
Coliphage f2	Silty sand Sand	183 5
Bacteriophage PRD-1, host <i>Salmonella typhimurium</i> LT2	Sand Sand Sand and gravel Fractured till Sand Sand Sand Fractured shale saprolite	12.5 4 40.5 4 2 30 8 18
Coliphage $\phi$ x174, host <i>E. coli</i> 13706	Sand and gravel Sand and gravel porous aquifer	38 40.5 100
Coliphage MS-2	Sand and gravel Gravel Fractured till Sand Sand Sand Fractured shale saprolite Sand Sandy loam over till	40.5 401 4 30 34 38 13.5 1.5 85
Coliphage T7	Sand and gravel	162.5
Coliphage —1	Sand	2
Coliphage Q-beta	Sandy loam over till	85
Coliphage T4	Karst limestone	1600
Coxsackievirus B3	Sand	408

Microorganism	Medium	Horizontal Distance (meters)
Echovirus	Coarse sand and fine gravel	45.7
Enterovirus	Sandy loam	14.5
Poliovirus	Medium sand	0.6
	Silt loam	46.2
	Medium to fine sand	9
	Loamy medium sand	6
	Coarse sand and fine gravel	3
	<b>Sand and gravel</b>	<b>19.4</b>

Source: Adapted from Yates and Yates (1988) and other studies as summarized below.

Some new data available since the publication of Yates and Yates (1988) are summarized below.

**Rossi et al. (1994)**—At a sand and gravel aquifer test site in Switzerland, Rossi et al. injected the bacteriophage T7 and f1 into the aquifer. Bacteriophage T7 were recovered 162.5 m from the injection well and bacteriophage f1 were recovered 112.5 m from the injection well.

**Bales et al. (1995)**—At a sand aquifer test site in Cape Cod, Massachusetts, Bales et al. reported that the bacteriophage PRD-1 were recovered 12 m from the injection well.

**Pieper et al. (1997)**—At a sand aquifer test site in Cape Cod, Massachusetts, Pieper et al. reported that bacteriophage PRD-1 were recovered 4 m from the injection well.

**Bales et al. (1989)**—At a sand aquifer near Tucson, Arizona, Bales et al. reported that bacteriophage f2 were recovered 5 m from the injection well.

**DeBorde et al. (1998)**—At a septic tank drainfield in a sand and gravel aquifer in Montana, DeBorde et al. reported that somatic coliphage were detected at a distance of 38 m from the injection site.

**DeBorde et al. (1999)**—At a sand and gravel aquifer test site in Montana, DeBorde et al. reported that the vaccine strain of poliovirus was recovered at a distance of at least 19.4 m and the bacteriophage (both somatic and male-specific) were recovered at a distance of 40.5 m.

**Sinton et al. (1997)**—At a gravel aquifer irrigation site, Sinton et al. reported that MS-2 coliphage were recovered at a distance of 401 m from the injection well site.

**Bales et al. (1997)**—At a sand aquifer test site in Ontario, Bales et al. reported that PRD-1 and M-1 coliphage were recovered at monitoring wells located 2 m from the injection well.

**McKay et al. (1993)**—At a fractured, clay-rich till site in Ontario, McKay et al. reported that MS-2 and PRD-1 were recovered in seepage collectors located 4 m from the infiltration trench.

**Oetzel et al. (1991)**—At a porous media aquifer in Germany, bacteriophage  $\phi$ x174 were recovered 100 m from the injection point.

**Schijven and Rietveld (1997); Schijven et al. (1999)**—At three well fields in sand aquifers in the Netherlands, Schijven and Rietveld and Schijven et al. report FRNA coliphage and bacteriophage transport of 30 m, 2 m, and 4 m.

**Schijven et al. (2000)**—At a deep well injection site in a sand aquifer in the Netherlands, Schijven et al., report MS-2 bacteriophage recovery at a distance of 38 m. Bacteriophage PRD-1 and *E. coli* WR1 were recovered at a distance of 8 m.

**Dowd and Pillai. (1997)**—At a sand aquifer research site in College Station, Texas, Dowd and Pillai report that MS-2 bacteriophage were recovered at a distance of 34 m.

**McKay et al. (2000)**—At a fractured saprolitic shale research site in Oak Ridge, Tennessee, McKay et al. report that MS-2 was recovered at a distance of 13.5 m and PRD-1 was recovered at a distance of 18 m.

**Higgins et al. (2000)**—At a septic tank research site in a sand aquifer in Cape Cod, Massachusetts, Higgins et al. report 3-log removal of MS-2 over a 1.5 m distance from a septic tank to sample pans buried beneath a leach trench.

**Curry et al. (1999)**—At six residential septic tank systems with monitoring wells, positive samples were detected at distances greater than 100 feet. PRD-1 was found at four of six sites; MS-2 at five of six sites; *B. subtilis* at five of six sites. Positive samples were found as long as 62-77 days after septic tanks were spiked. It is possible that indicators could have been found at even further distances and longer times of travel if the study had continued.

### **4.3 Factors Affecting the Fate and Transport of Bacteria in the Subsurface**

This section describes the hydrogeologic features that govern the transport and survival of bacteria in the subsurface. As with viruses, bacterial survival and transport in the subsurface varies for differing types of bacteria and is dependent on a variety of factors including (adapted from Yates and Yates 1988):

- Light
- Temperature
- Hydrogeologic conditions
- Soil properties, including mineral coatings on grains
- pH
- Inorganic ions/salt species and concentration
- Organic matter

- Microbial activity
- Iron content
- Moisture content
- Oxygen (for aerobic bacteria), electron donors (for anaerobic bacteria)
- Nutrient content

The transport and persistence of a bacterium in the subsurface is affected by most of the same parameters governing virus transport (discussed above). In comparison to bacteria, viruses are longer lived in ground water and saturated and unsaturated soils than bacteria. Arnade (1998) sampled approximately 50 wells in Florida and reported that twice as many fecal coliforms were found in ground water samples collected at various distances (12 to over 36 meters) from septic tanks during the Florida wet season as compared to the dry season. Fattal et al. (1984) compared the relative survival of enteroviruses, total coliform (TC), fecal coliform, and fecal streptococci and concluded that enteroviruses are more resistant to hostile environmental conditions. Comparison among bacteria suggests that enterococci survive longer than coliforms (McFeters 1974). Filip et al. (1988) report that *E. coli* survived for up to 100 days in 10° C ground water. Kudryavtseva (1972) determined that coliform and *E. coli* were viable in ground water for 3.5 to 5.5 and 3 to 4 months, respectively.

One additional important parameter may be oxygen and nutrient content. Because of the important role of bacteria in enhancing the remediation of ground water contaminated by organic chemicals, a large amount of research has been undertaken recently to evaluate the feasibility of injecting bacteria into the subsurface. A key issue is whether there are sufficient nutrients and oxygen or electron donors to allow the bacteria to thrive and be transported through the subsurface. These data are not summarized here but are important to evaluating bacterial fate and transport in the subsurface. Recent papers summarize much of the recent literature and important issues associated with bacterial transport for soil, (Schafer et al. 1998) unsaturated sand (Schafer et al. 1998), and saturated sand (Hendry et al. 1999; Bolster et al. 2000; Brown et al. 2002).

Under hydrogeologic conditions where there are reduced opportunities for contact between the bacteria and the soil, sediment, and rock particles, pathogens and other microorganisms are transported more efficiently and for longer distances. Therefore, despite the complexities associated with the interactions among bacteria, water, and the solid materials in the subsurface environment, bacterial transport, like viral transport (see section 4.2), over significant distance is most likely in hydrogeologic settings such as conduits in karst aquifers, fractures in fractured bedrock aquifers, and large pore openings in gravel aquifers (herein defined as sensitive aquifers). The available data on bacterial transport distances was summarized by Yates and Yates (1988) and are presented in Exhibit 4.3. Since the publication of Yates and Yates (1988), several studies have documented bacterial transport distances. New data (post-1988) in Exhibit 4.3 are shown in bold. The more significant studies are also summarized in the text that follows.



### Exhibit 4.3 Migration of Bacteria in the Subsurface

Microorganism	Medium	Horizontal Distance (meters)*
<i>Bacillus stearothermophilus</i>	Fractured rock	29
<b><i>Bacillus subtilis</i> endospores</b>	<b>Sandy gravel</b>	<b>90</b>
Bacteria	Fine sand Medium to coarse sand Alluvial gravel Pea gravel and sand Coarse gravel Gravel Sandy clay Fine to coarse sand Fine to medium sand <b>Medium to coarse sand with some gravel</b> <b>Sand</b>	457 21 0 30 457 920 15.25 30.5 6.1 <b>12</b> <b>6</b>
<i>Clostridium welchii</i>	Fine and medium sand	15.5
<b><i>Clostridium bifermentans</i></b>	<b>Sand</b>	<b>38</b>
Coliform bacteria	Sand and gravel Fine sandy loam Fine sand Pebbles Weathered limestone Coarse sand and gravel Sandy clay loam Sandy clay loam Sandy loam	850 1.2 2 850 1000 (fracture flow) 55 6.1 13.5 28
<i>Escherichia coli</i>	Sand Fine and coarse sand Fine and medium sand Sand and sandy clay Silt loam Silty clay loam Medium sandy gravel Fine sandy gravel with cobbles Silty clay loam Fine sand Fine sand <b>Gravel</b> <b>Fractured granite</b> <b>Karst limestone</b>	3.1 24.4 3.1 10.7 3 1.5 125 50 15 19.8 70.7 <b>401</b> <b>13</b> <b>200</b>

Microorganism	Medium	Horizontal Distance (meters)*
<b><i>Escherichia coli</i> WR1</b>	<b>Sand Porous sandy</b>	<b>8 236</b>
Fecal coliform bacteria	Fine loamy sand and gravel Stoney silt loam Fine to medium sand Gravel with sand and clay Gravel	9.1 900 2.4 9 42
<i>Salmonella typhi</i>	Limestone	457
<b><i>Serratia marcescens</i></b>	<b>Porous media</b>	<b>25</b>
<i>Streptococcus faecalis</i>	Silty clay loam	0.55
<i>Streptococcus zymogenes</i>	Sandy gravel	15.2

\*Ranges are estimates.

Source: Adapted from Yates and Yates 1988 and other studies as listed below.

New data available since the publication of Yates and Yates (1988) are summarized below:

**Pang et al. (1998)**—At a sandy gravel experimental site in New Zealand, Pang et al. reported *Bacillus subtilis* endospore transport of 90 m from the injection well.

**Bales et al. (1995)**—At a sandy aquifer test site in Cape Cod, Massachusetts, Bales et al. reported bacterial transport of 12 m from the injection well.

**Harvey et al. (1993)**—At a sandy aquifer test site in Cape Cod, Massachusetts, Harvey et al. reported bacterial transport of 6 m from the injection well.

**Sinton et al. (1997)**—At a gravel aquifer irrigation site in New Zealand, Sinton et al. reported *E. coli* J6-2 was recovered 401 m from the injection well.

**Champ et al. (1988)**—At a fractured granite test site in Chalk River, Canada, Champ et al. reported *E. coli* recovery at a distance 13 m from the injection well.

**Oetzel et al. (1991)**—At a porous media test site in Germany, Oetzel et al. reported *Serratia marcescens* transport of 25 m from the injection well.

**Orth et al. (1997)**—At a confined karst limestone test site in Germany, Orth et al. reported *E. coli* transport of 200 m in 2-5 days.

**Schijven et al. (2000)**—At a deep well injection site in a sand aquifer in the Netherlands, Schijven et al. report *Clostridium bifermentans* recovery at a distance of 38 m. *E. coli* WR1 were recovered at a distance of 8 m.

**Arnade (1999)**—At a porous sand Florida site, *E. coli* was found in ground water wells at distances greater than 36 m from septic tanks.

One study (Sinton et al. 1997) directly injected both bacteriophage (MS-2), dye (rhodamine WT) and bacteria (*E. coli* J6-2) into the saturated zone, so the comparative mobility of a bacterium and a bacteriophage could be established for one site. The study aquifer is comprised of alluvial gravel, and the distances between injection site and the two monitoring wells were 385 and 401 m. The bacteria and viruses were not co-injected, but were injected separately, 48 hours apart. All travel times were corrected for this lag period. For this tracer injection experiment, the time to peak concentration at 385 m was 51 hours for the viruses and 57 hours for the bacteria. At 401 m, the virus peak arrived in 57 hours and the bacteria peak arrived in 67 hours. Additional information on the use of microorganisms as tracers in ground water injection and recovery experiments can be found in a review of published work by Harvey (1997) and Harvey and Harms (2001).

#### 4.4 Conditions At and Near the Wellhead

The purpose of this section is to describe the factors that may allow microbial contaminants to bypass the naturally protective features of the subsurface and take a more direct path from the surface to the well intake point. Under normal ground water flow conditions, the soil acts as a protective barrier and ground water flow is slow enough that microbial contaminants become inactive long before they reach a PWS well intake point. However, conditions near the wellhead may act to allow microbial contamination to bypass the protective soil or to increase substantially the ground water flow rate so that microbial contaminants remain infectious when they reach the intake.

Ground water contamination may occur at the wellhead in several ways. The main causes are poor well location and/or construction, improperly abandoned wells, the nearby presence of test holes, monitoring wells or exploratory wells, and location of a well within an area of intense ground water development, as described below:

- **Poor Well Location and/or Construction**—A water supply well should not be located downgradient of a possible contaminant source or in a low-lying area where it is susceptible to flooding. In addition, an improperly constructed water supply well may allow surface runoff or surface waters to enter the well because the well seal is non-existent, compromised, or broken. A shallow well may be particularly vulnerable to surface water and generally should be cased and grouted to the well screen. If the integrity of the well seal is compromised, aerobic bacteria can thrive in the gravel pack surrounding a well casing or screen. Typically, these bacteria are only non-pathogenic bacteria, but the gravel pack can harbor known pathogens as well. Regular surface inspection is necessary to examine the visible barriers acting to protect the integrity of the well seal.

Although guidelines exist for the construction of water supply wells, these guidelines may not always be adhered to during the installation of some wells; in particular, wells

constructed for non-potable use such as irrigation or livestock wells. If these wells penetrate the same aquifer as a nearby PWS well, their poor construction may allow contaminants to enter the common ground water source. Furthermore, since many old wells were constructed before the institution of more rigorous well construction guidelines, greater care should be taken when evaluating the potential contamination scenarios for these wells.

- **Abandoned Wells**—Historically, well abandonment and plugging have not been properly planned, designed, and executed (USEPA 1990; Canter et al. 1987). The well casing is typically removed if it is not too worn or corroded, thereby removing an important protective element if the well is not adequately plugged. Occasionally, abandoned wells and mines have also been used as disposal sites for a variety of wastes. Such wells would then serve as a direct conduit for contaminated ground water to spread to other zones within an aquifer, or allow contaminants to enter adjacent aquifers at lower potentiometric elevation (lower head or fluid pressure) (USEPA 1990).
- **Test Holes, Exploratory Wells, and Monitoring Wells**—Many test holes and exploratory wells have been dug or drilled into the subsurface over time for excavation design, industrial minerals such as limestone, sand, and gravel, or economically valuable deposits of oil, gas, coal, ore, and water. Other shallower wells include soil boreholes and seismic shot holes. Where these holes are not backfilled, or are poorly constructed or improperly abandoned, they add to the number of potential conduits for contaminated water to enter ground water aquifers.
- **Ground Water Development**—When a new well is installed, the pumping at the new well may draw down, or redirect, adjacent ground water flow. Changes in flowpaths can also result in increased flow rates. Such changes may cause leakage between aquifers or induce infiltration of surface water into the ground water source (USEPA 1990). Further pumping may pull contaminated ground water into the new well and/or provide a continuous means for contaminants to enter the aquifer tapped by other water supply wells.

Contamination pathways unique to some hydrogeologic settings, such as karst, are especially important. The leakage and subsequent collapse of sewage treatment lagoons into a karst sinkhole as occurred in West Plains, Missouri, in 1978, (Craun 1984) in Lewiston, Minnesota, in 1991, and in Altura, Minnesota, in 1974 and 1976 (Jannik et al. 1991) suggest that sinkholes are an important pathway for microbial contamination of wells in karst hydrogeologic settings. In the West Plains event, it is reported that domestic wells were contaminated and 759 illnesses resulted.

Malard et al. (1994) found that bacteria traveled vertically along fractures from surface water to ground water in a karstified, fractured limestone and reached the ground water in about 2 hours. They conclude that “because of fracturing and especially of karstification, the potential for movement of bacterial contaminants through limestone rock is probably higher than in any other geological formations.” Malard et al. note that a relatively small number of preferential flow pathways are developed that can result in rapid percolation through the unsaturated zone. During high rainfall periods, the bacteria may regularly be flushed down to the saturated zone by quick percolation. They note that bacteria at one of their monitored sites had high occurrence rates at about 1 year after significant surface pollution had ceased. They suggested that bacteria could have reproduced or that they entered a “survival state” during harsh conditions.

Mahler et al. (2000) found high (60,000 colony forming units (CFU) per 100 mL) concentrations of fecal coliform and enterococci bacteria in karst monitoring wells near a wastewater irrigation site also studied by Malard et al. (1994). In particular, bacteria were found soon after rainfall, and there was an association of bacteria with suspended particles. Mahler et al. conclude that samples collected at 3 to 4 hour intervals were necessary to describe the breakthrough of bacteria in a karst monitoring well; lesser frequencies may be insufficient. Celico et al. (2004) found that fecal enterococci levels in a small karst aquifer, where grazing occurred as little as 250 m from the sampled spring, depended on the distribution of rainfall. When rainfall occurred all in 1 day, enterococci levels peaked 2 days later (at 9 CFU/100 mL). When rainfall was spread out over 3 days with breaks in the precipitation, enterococci levels peaked 2 days later (at more than 30 CFU/100 mL), declined for 2 days, and then increased and peaked a second time (near 25 CFU/100 mL) 6 days after the precipitation first occurred. In two recent papers, Gunn et al. (1997) and Tranter et al. (1997) found high fecal coliform (Gunn et al., Tranter et al.) and high fecal streptococci (Gunn et al.) in karst springs. Both papers conclude that in these sites, soil cover over karst is unable to reduce fecal contaminants to insignificant levels. In a study of 50 domestic wells in a karst region of Berkeley County, West Virginia, Mathes (2000) found 31 (62 percent) positive for TC, 16 (32 percent) positive for *E. coli*, and 15 (30 percent) positive for fecal coliform bacteria. Personne et al. (1998) studied *E. coli* and enterococci over an 8-km distance in a karst aquifer. High bacteria concentrations were found as the result of rainfall-induced high water levels in the aquifer. The maximum travel time was about 20 days.

Public health protection may require additional measures in karst areas that are not needed elsewhere. At a minimum, sanitary survey inspectors must be especially vigilant in karst areas because the normally protective soils may be readily bypassed by contaminant runoff or leakage into a sinkhole. Source water protection measures used in other hydrogeologic settings (e.g., determining the area contributing ground water to a pumping well) are not applicable in karst areas because the ground water flow is too rapid and flow occurs only in conduits.

Where soils are thin or absent, open fractures in bedrock aquifers may not be completely filled with soil and, thus, open directly onto the ground surface. In these hydrogeologic settings, open fractures act similarly to sinkholes, albeit with much smaller openings into the aquifer. Nevertheless, the fracture openings are likely large enough to transmit viruses and bacteria if they are not clogged by soil and sediment. Thus, surface runoff or seepage leakage can be transmitted directly into the aquifer. De Serres et al. (1999) reported hepatitis A virus (HAV) detection by polymerase chain reaction (PCR) in a private well in fractured bedrock (with soil and colluvial overburden) at the end of January, about 7 months after the adjacent cesspool was emptied of sludge and the residence was vacated.

In summary, well design, location, and wellhead protection have an important role in preventing public health risk to PWS consumers. Any natural (e.g., sinkhole or open vertical fracture) or man-made (e.g., unplugged abandoned well) feature that allows microbial contamination to reach the well and bypass the naturally protective soil/sediment barrier can pose a hazard to a humans consuming drinking water from that source.

#### **4.5 Waterborne Disease Outbreaks Resulting from Subsurface Microbial Transport to PWS Wells in Sensitive Aquifers**

The purpose of this section is to describe the public health risk associated with microbial pathogen transport through the subsurface to a PWS well. As discussed above, the interactions within the subsurface that govern the fate and transport of microbial contaminants are complex. Where ground water flows at a high rate and in large volume, features such as conduits in karst aquifers, fractures in fractured bedrock aquifers, and large pores in gravel aquifers (these types of aquifers are defined by the GWR as sensitive aquifers) minimize the opportunities for microorganisms to come in contact with the conduit, fracture, or pore walls (surfaces). The fast ground water velocity provides insufficient time for significant virus inactivation in the subsurface. As a result, the microorganisms are capable of being transported for longer distances. The data from waterborne disease outbreaks support this working hypothesis. In the following discussion, available data on waterborne disease outbreaks in sensitive aquifers are compiled to illustrate the pathogen transport capability of these aquifers. It should be noted that many waterborne disease outbreaks are not recognized, or if recognized, not investigated sufficiently, to determine the site hydrogeologic setting and the role that sensitive aquifer conditions may contribute to the outbreak event.

Karst aquifers are particularly sensitive because they may have both vertical and horizontal conduits, and some conduits can be exceptionally large (sinkholes and caves). Public health workers have published detailed reports about waterborne disease outbreaks that occur, in part or largely due to the PWS location in a karst hydrogeologic setting. The purpose of these publications was likely to alert other public health professionals and water purveyors of the need to be especially vigilant in protecting water supply wells located in karst aquifers because of their intrinsic sensitivity to microbial (and other types of) contamination. CDC lists karst as a parameter to be identified if data are available on the outbreak reporting form. Exhibit 4.4 lists the published (and two unpublished) reports of waterborne disease investigations in which the karst hydrogeologic setting was an important contributor to the outbreak.

Fractured bedrock aquifers are composed of a network of typically interconnected fractures ranging from vertical to horizontal orientation that can readily transmit fecal contamination. Contaminant transport may be enhanced if soils are thin or absent and the fracture openings are not clogged by soil or sediment. Published scientific reports by public health workers on waterborne disease outbreaks have noted the role that the fractured bedrock contributed. For example, Lawson (1991) writes that “effluent from the resort’s sewage treatment facility seeped through the fractures in the subsurface rock (with little filtration) directly into the resort’s deep well (150-200 m).” This outbreak occurred in northern Arizona, an arid area where soils are typically not well developed. As with karst aquifers, the sensitive characteristics of fractured bedrock aquifers minimize the opportunities for microbial contaminants to come in contact with the aquifer materials, and thus, allow pathogens to travel greater distances. The fast ground water velocity provides insufficient time for significant virus inactivation in the subsurface. Exhibit 4.5 lists the published and unpublished reports of waterborne disease investigations in which the fractured bedrock aquifer setting was an important contributor to the outbreak.

Gravel aquifers are not particularly numerous, as compared with sand and gravel aquifers. Fewer PWSs use these aquifers and so any reports of outbreaks are correspondingly limited. No data are available that specifically implicate a gravel aquifer as having a significant role in a published waterborne disease outbreak.

### Exhibit 4.4 Waterborne Disease Outbreaks Reported in Karst Hydrogeologic Settings

Location	Reference	Number of Illnesses/Agent
Richmond Heights, FL	Weisman et al. 1976	1,200 cases/ <i>Shigella</i>
Cabool, MO	Swerdlow et al. 1992	243 cases/ <i>E. coli</i> O157:H7; 4 deaths
Georgetown, TX	Hejkal et al. 1982	8,000 cases/Coxsackievirus; 36 cases/HAV
Braun Station, TX	D'Antonio 1985	251 cases/Norwalk virus
Henderson County, IL	Parsonnet et al. 1989	72 cases/unknown
Lancaster, PA	Bowen and McCarthy 1983	49 cases/HAV
Racine, MO	MO Department of Health, unpublished report 1992	28 cases/HAV
Buttermilk Falls spring, Meade County, KY	Bergeisen et al. 1985	73 cases/HAV
Walkerton, Ontario	Golder Associates 2000; Health Canada 2000	1,346 cases/ <i>E. coli</i> O157:H7 (+ <i>Campylobacter</i> ); 6 deaths
South Bass Island, OH	Ohio EPA 2005, CDC 2005	1,450 cases/ <i>Campylobacter</i> , norovirus, <i>Salmonella typhimurium</i> , and unknown

## Exhibit 4.5 Waterborne Disease Outbreaks Reported in Fractured Bedrock Aquifers

Location	Reference	Number of Illnesses/Agent
Coeur d'Alene, ID	Rice et al. 1999	117 cases/ <i>Arcobacter butzleri</i>
Island Park, ID	CDC 1995	82 cases/ <i>Shigella</i>
Drummond Island, MI	Ground Water Education in Michigan 1992	39 cases/Unknown
Northern AZ	Lawson et al. 1991	900 cases/Norwalk virus
Big Horn, WY	Anderson et al. 2003	35 cases/Norwalk virus
Central WY	Parshionikar et al. 2003	84 cases/norovirus
Harford County, MD	Harford County Dept. of Health, unpublished data, 2006; personal communication with Bill Banks, U.S. Geological Survey Water Resources Division; and Kellogg Schwab, Johns Hopkins School of Public Health, 2006	200 cases/norovirus (septic tank is possible source)

### 4.6 Conclusions

Many factors apparently control the introduction, transport, persistence, and removal of viruses and bacteria in subsurface media. Because these factors are often interlinked and interrelated, defining the processes involved in the survival and migration of viruses and bacteria is a complex task. Factors such as pH, ionic strength, virus, and soil types affect pathogenic adsorption to soils, sediments, and rock surfaces. In addition, these factors likely have a direct or indirect effect on pathogen survival. Fractures, conduits, and sinkholes, as well as wellhead deficiencies, enable relatively unimpeded microbial transport. Aquifer and well construction characteristics that slow the movement of these organisms reduce the likelihood they will reach the ground water supply due to adsorption, natural die-off, or predation by other organisms.

A review of the reported data on virus and bacterial transport in the subsurface suggests that, despite the variability among experimental sites and methods and among test organisms, sand and gravel aquifers are capable of allowing virus and bacterial transport over long distances in short time periods. The data for virus and bacteria transport in karst and fractured bedrock aquifers, as compared to sand and gravel aquifers are fewer, but lead to some general conclusions. The high ground water flow velocity and direct flow paths in karst and fractured bedrock aquifers illustrate the sensitivity and perhaps highlight the vulnerability of these aquifers to fecal contamination. Outbreak reports published in the literature have focused on the role of sensitive aquifers in contributing to waterborne disease outbreaks. These reports have established that both karst and fractured bedrock aquifers have been important contributors to outbreak events.



## 5.0 Microbial Contaminant Monitoring Methods

### 5.1 Introduction

This chapter introduces the analytical methods to be used by ground water public water systems (PWSs) that have a total coliform-positive sample, and for any other Ground Water Rule (GWR) source water monitoring required by the state. It also discusses some of the methods used in the key studies presented in Chapter 6. Under the GWR, a system must monitor for fecal contamination rather than for pathogens, using one of the following fecal indicators: *E. coli*, enterococci, somatic coliphage, or male-specific coliphage, as required by the State.

Exhibit 5.1 lists the microbial methods that must be used for source water monitoring under the GWR. Section 5.2 describes the EPA-approved methods for identifying bacterial indicator organisms for the GWR, as well as methods used in a number of the occurrence studies in Chapter 6. Section 5.3 describes the methods used to identify coliphage. Section 5.4 describes viral pathogen monitoring analyses used in key studies. Indicator monitoring strategy and rationale are discussed in Chapter 2 and the GWR preamble. Appendix A briefly summarizes viral pathogen methods under development and in use by some researchers.

**Exhibit 5.1 Analytical Methods for Source Water Monitoring for the GWR**

Indicator	Method <sup>1,2</sup>
<i>E. coli</i>	Colilert Test (Standard Method 9223B) Chromogenic Substrate
	Colisure Test (Standard Method 9223B) Chromogenic Substrate
	Membrane Filter Method with MI Agar (EPA Method 1604)
	m-ColiBlue24 Test
	E*Colite Test
	EC-MUG (Standard Method 9212F; SMWW, 20th ed.) or the NA-MUG (Standard Method 9222G, SMWW, 20th ed.) as a confirmation step after the Multiple-Tube Fermentation (Standard Method 9221A,B,C,D); Membrane Filter Technique (Standard Method 9222 A,B,C)
Enterococci	Multiple-Tube Technique (Standard Method 9230B)
	Membrane Filter Technique (Standard Method 9230C/EPA Method 1600)
	Enterolert
Coliphage	Two-Step Enrichment Presence-Absence Procedure (EPA Method 1601)
	Single Agar Layer Procedure (EPA Method 1602)

<sup>1</sup> The time from sample collection to initiation of the analysis may not exceed 30 hours. Systems are encouraged, but not required, to hold samples below 10° C during transit.

<sup>2</sup> Sample volume is 100 mL for all fecal indicators.

## 5.2 Monitoring Bacterial Indicators of Fecal Contamination

The following are brief descriptions of methods used in a number of occurrence studies in Chapter 6, including detection methods for the bacterial indicators found in fecally-contaminated ground water.

### 5.2.1 Total Coliforms

The coliform group consists of all aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose. Total coliforms (TC) are used (1) to determine treatment efficiency and (2) to assess integrity of the distribution system. Thus, under the final GWR, total coliforms are used to assess the vulnerability of a system to fecal contamination and do not necessarily indicate whether the system is contaminated by fresh fecal contamination.

Total coliform monitoring of the source water is not required under the GWR, but coliform methods are discussed here because several occurrence studies in Chapter 6 tested for total coliform. Several methods have already been approved under the Total Coliform Rule (TCR) and/or the Surface Water Treatment Rule (SWTR). These methods require a 100 mL sample volume. Some of these tests may also be used for *E. coli* detection. Brief descriptions of these methods are provided below:

***Multiple-Tube Fermentation Technique for Members of the Coliform Group (Standard Method 9221 A,B,C,D, APHA 1998)***: This test is performed by inoculating several tubes containing a growth media with the sample water. The tubes are then incubated and subsequently checked for the presence of acidic growth or gas. Tubes containing acidic growth or gas are verified for the presence of coliforms by additional bacteriological testing. Bacterial density, if needed, is then estimated based on the number of positive and negative tubes.

***Membrane Filter Technique for Members of the Coliform Group (Standard Method 9222 A,B,C, APHA 1998)***: This test is performed by filtering samples through a sterile membrane filter using a filtration unit. As the sample is filtered, the coliform bacteria are all retained by the filter. The membrane filter is then transferred to the surface of a selective (for total coliforms) semi-solid growth medium in a petri dish and incubated. At the end of the incubation period, the presence, or if needed, the density, of coliform colonies is determined.

***Membrane Filter Method MI Agar (EPA Method 1604, USEPA 2002a)***: This is similar to other membrane filter methods in procedure, but uses a membrane filter agar medium containing chromogen (a chemical that changes color), indoxyl-beta-D-glucuronide, and a fluorogen (a chemical that fluoresces). It was developed to simultaneously detect and enumerate *E. coli* and total coliform in water samples on the basis of their enzyme activities.

***Chromogenic Substrate Test (also Colilert and Colisure) (Standard Method 9223 B, APHA 1998)***: Colilert is a presence-absence test conducted by adding reagent to a sample that is either retained in the container or poured into a tray for incubation for 24 hours. Specific enzymes produced by coliforms and *E. coli* alter the color and/or the fluorescence of the water. Colisure is a similar test that produces definitive results at 24 hours.

### 5.2.2 *E. coli*

All the *E. coli* tests below detect total coliforms and *E. coli* simultaneously or sequentially. All have been approved for use under the TCR at 40 CFR 141.21(f). The false-positive rate and false-negative rate for each *E. coli* test were addressed in Federal Register notices associated with the TCR.

#### 5.2.2.1 Enzyme Substrate Methods

The Colilert test, Colisure test, and E\*Colite test simultaneously determine the presence of total coliforms and *E. coli*. The tests are based on the detection of two enzymes, beta-D-galactosidase and beta-D-glucuronidase, that are characteristic of the TC group and *E. coli*, respectively. Beta-D-galactosidase acts upon a chromogenic enzyme substrate in the medium (chlorophenol red beta-galactopyranoside for Colisure, X-GAL for E\*Colite, and o-nitrophenyl-beta-D-galactopyranoside for Colilert), producing a color change. Any beta-D-glucuronidase produced hydrolyzes (reacts with water) 4-methylumbelliferyl-beta-D-glucuronide (MUG) in the medium, which fluoresces when exposed to ultraviolet light.

The Colilert and Colisure test involve the addition of a 100 mL drinking water sample, either as a single volume or as five 20-mL volumes, to a specially formulated dehydrated medium. After the specified incubation period at 35° C, the tube or bottle is examined for a color change and fluorescence. These two tests are described more fully in *Standard Methods* (20<sup>th</sup> edition), Section 9223B.

The E\*Colite test also involves a dehydrated medium to which a 100-mL water sample is added. The test consists of a packaged sterile burst-a-seal bag divided into three compartments. The upper compartment is used for sample collection and optionally contains a sodium thiosulfate tablet to eliminate free chlorine and/or bromine in the water. The middle compartment of the bag contains the medium for growth and enzyme substrates for detection of total coliforms and *E. coli*. The lower compartment optionally holds a bactericide (a quaternary amine) that the analyst can introduce to kill the replicating coliforms. First, a 100-mL water sample is added to the upper compartment and the bag sealed. Then the water sample is pushed through the burst-a-seal into the medium, and the two are mixed. The bag is then incubated for 28 hours at 35° C and observed for the presence of blue/green color (total coliforms) or fluorescence (*E. coli*).

#### 5.2.2.2 Membrane Filter Methods

The m-ColiBlue24 test is a membrane filtration method that simultaneously determines the presence or absence of total coliforms and *E. coli*. TC colonies growing on the lactose-based medium are identified by a nonselective dye, 2,3,5-triphenoltetrazolium chloride (TCC), which produces red colonies. The selective identification of *E. coli* is based on the detection of the beta-glucuronidase enzyme. The test medium includes the chromogen 5-bromo-4-chloro-3-indoxyl-beta-D-glucuroindice (BCIG) which is hydrolyzed by the enzyme, releasing an insoluble indoxyl salt that produces blue colonies (*E. coli*). The test involves filtering a 100-mL drinking water sample through a membrane filter, which is transferred to a petri plate containing an absorbent pad saturated with m-ColiBlue24 broth. After incubation at 35° C for 22±2 hours, the membrane is examined for colony growth.

MI Agar is a membrane filtration method that, analagous to the Colilert and Colisure tests, is based upon the detection of two enzymes, beta-D-galactosidase and beta-D-glucuronidase. TCs produce the enzyme beta-galactosidase which hydrolyzes the 4-methylumbelliferyl-beta-D-galactopyranoside in

the medium to form a product which fluoresces. *E. coli* produces beta-glucuronidase, which hydrolyzes indoxyl-beta-D-glucuronide to form a blue color. Another format, MI broth, may become more commercially available later. MI Agar is described by Brenner et al. (1993) and in EPA Method 1604 (USEPA 2002a).

### 5.2.2.3 *E. coli* Tests Where Total Coliform Detection is an Intermediate Step

The EC-MUG test and Nutrient Agar-MUG test are methods that use a TC-positive culture, rather than a water sample, to confirm *E. coli*. Thus, under the GWR, these two tests are used only when a total coliform test is conducted first (i.e., TC testing is an intermediate step in this method). The EC-MUG sample may be inoculated from a TC-coliform-positive culture from one of the following methods previously described for TC: Standard Total Coliform Fermentation Technique (*Standard Methods* 9221B), Presence-Absence (P-A) Coliform Test (*Standard Methods* 9221D), Standard Total Coliform Membrane Filter Procedure (*Standard Methods* 9222B), or any other approved membrane filtration method if the filter contains *E. coli*.

EC Medium is a fermentation test described by *Standard Methods* (Section 9221E) for fecal coliforms. The addition of 50 ug/mL MUG to EC medium provides specificity for *E. coli*. After being inoculated with a total coliform-positive culture, EC-MUG medium is incubated at 44.5°C for 24 hours, and examined under an ultraviolet (UV) light for fluorescence. Fluorescence indicates the presence of *E. coli* (*Standard Methods*, 19<sup>th</sup> and 20<sup>th</sup> editions, 9221F).

Nutrient agar (*Standard Methods* 9221B) is a common medium for heterotrophic bacteria. The addition of 100 ug/mL MUG to nutrient agar provides specificity for *E. coli*. After being inoculated with a TC-positive culture, nutrient agar-MUG medium is incubated at 35°C for 4 hours, and colonies are examined under a UV light for fluorescence. Fluorescence indicates the presence of *E. coli*.

### 5.2.3 Enterococcus

The enterococcus group consists of several species of the bacteria in the genus *Streptococcus* that typically live in the gut of warm-blooded animals. EPA has approved the following methods for use under the GWR to detect enterococci (and a closely-related group, the fecal streptococci).

**Multiple-Tube Technique (Standard Method 9230 B, APHA 1998):** This method consists of inoculating tubes containing media optimal for streptococci growth (azide dextrose broth) with the sample water. The tubes are incubated and then checked for turbidity. Tubes displaying turbidity are then verified for the presence of enterococci by plating on Pfizer selective enterococcus (PSE) agar.

**Membrane Filter Techniques (Standard Method 9230 C, APHA 1998 or EPA Method 1600, EPA 2002b):** This method involves taking a sample and filtering it through a sterile membrane filter. The filter is then transferred to a selective growth medium within a petri dish and incubated. After incubation, the presence of typically shaped and colored enterococcus colonies are determined.

**Enterolert (Budnick et al. 1996; ASTM Method #D6503-99):** The Enterolert test is an enzyme substrate method. Enterolert reagent is added to a 100-mL water sample, and incubated at 41°C±0.5°C for 24-48 hours. Fluorescence under a UV lamp indicates the presence of enterococci.

### 5.3 Monitoring Viral Indicators of Fecal Contamination

Coliphage are viruses that infect *E. coli*. Because *E. coli* are closely associated with fecal contamination, coliphage can also be assumed to be associated with such contamination. As discussed in Chapter 2, there are two categories of coliphage - somatic coliphage and male-specific (also known as F+ or F-specific coliphage). Somatic coliphage enter a bacterial cell through the main cell membrane, while male-specific enter through the pili, projections from the bacterium that are used to exchange genetic material with other bacteria. Different strains of *E. coli* are used for each type of coliphage, although sometimes both kinds of phage can infect the same strain of *E. coli*. Neither category of coliphage is pathogenic to humans.

***Male-Specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure (EPA Method 1601, EPA 2001d)***: This test is a two-step procedure that could potentially be used as a quantitative method, but was validated by EPA only as a qualitative presence-absence test. A sample of at least 100 mL ground water is supplemented with magnesium chloride. The host bacteria are log-phase *E. coli* F<sub>amp</sub> for F+ coliphage and *E. coli* CN-13 for somatic coliphage. First, tryptic soy broth (TSB) is used to enrich the male-specific (F+) and somatic coliphage. After an overnight incubation, samples are “spotted” onto a lawn of host bacteria specific for each type of coliphage, incubated, and checked for circular lysis zones that indicate the presence of coliphage.

***Male-Specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (EPA Method 1602, EPA 2001e)***: This method is a quantitative procedure that uses a single agar layer technique for determining coliphage density in ground water samples. It requires at least a 100 mL sample to which magnesium chloride, the host bacteria, and an equal volume of double-strength molten tryptic soy agar medium are added. The host bacteria are log-phase *E. coli* F<sub>amp</sub> for F+ coliphage and *E. coli* CN-13 for somatic coliphage. The total volume of the mixture is poured onto 5 to 10 plates and incubated overnight. All plates from a single sample are examined for plaque formation (circular lysis zones of bacterial host lawn clearing where each plaque represents one bacterium initially infected by a phage). The quantity of coliphage in a sample is expressed as plaque forming units (PFU/100 mL). For quality control purposes, both coliphage-positive reagent water and a negative reagent water sample (method blank) are analyzed for each type of coliphage with each sample batch.

***Coliphage Detection (Standard Method 9224, APHA 1999)***: This method is not EPA-approved for GWR monitoring, but was used in a number of key studies. The method requires a viable population of the *E. coli* host for the coliphage to infect and an agar surface for both to grow. The host bacteria are induced to grow as a monolayer of living bacteria. The presence of coliphage is apparent with the formation of circular clear areas (plaques) on the agar surface. These are areas in which most of the host have been infected and killed by the invading viruses. The number of plaques is used to calculate the coliphage density. Somatic and male-specific coliphage can be differentiated by the type of *E. coli* host used.

### 5.4 Monitoring Enteric Viral Pathogens

The GWR approach uses a monitoring scheme for fecal indicators, not pathogens. However, pathogen monitoring is described briefly because cell culture was used in a number of key studies presented in Chapter 6.

Cell culture of enteric viruses offers the unique advantage of isolating an infectious viral pathogen, rather than an indicator that may or may not correlate with the occurrence of pathogens. The

disadvantage of this method is that many viral pathogens replicate only in their host organisms and are not culturable in cell culture, so some viruses will go undetected if only this method is used. In addition, viral pathogens may occur only sporadically in contamination sources (e.g., septic tanks) because few people may contribute to a source, few are infected at any particular time, and shedding occurs for only a short period (typically several weeks). Current pathogen monitoring tools, where available, are substantially more labor intensive, more expensive, and require more sophisticated expertise than monitoring for indicators (Organisation for Economic Co-Operation and Development 1999).

Cell culture for enteric viruses is based on the same principles as methods used for detecting coliphage. However, mammalian cells, rather than bacteria, are used as host cells. Cell culture methods detect viral pathogens that reproduce well in specialized cells (Buffalo Green Monkey kidney cells; BGM). The BGM cell line is optimized for polioviruses, coxsackieviruses, and echoviruses in drinking water. BGM also detects reovirus, which can include the pathogenic rotavirus, a member of the reoviridae family. The BGM cell line is not capable of detecting other pathogenic viruses, such as hepatitis A, and norovirus. To test for different viruses, multiple cell lines must be maintained, different growth media must be bought and stored, and other procedures such as reverse transcriptase polymerase chain reaction (RT-PCR) or integrated cell culture PCR (ICC-PCR) used (see Appendix A) (Lieberman 2002; Fout et al. 2003).

Analyses using cell cultures perform better with specific viruses that reproduce well at pH conditions specified in the method. Poliovirus, a key virus for which the method was originally developed, is exceptionally resistant to pH changes compared to other viruses. The poliovirus strain used is the weakened strain used in vaccine; it can sometimes be detected in contaminated ground water because children shed the virus in their stool after vaccination. Meaningful data using cell culture methods may not be available for other viruses that are less resistant to pH extremes (Grabow 2001).

Cell culture alone does not allow identification of the virus causing plaque formation. Cell culture should be followed by PCR, or other methods, to specifically identify the type of viruses present. PCR methods may also be used to identify enteric viruses (see Appendix A) but are not standardized. PCR methods alone cannot identify infectious agents. Instead, PCR identifies segments of genetic material that may or may not be associated with a living microorganism or active virus.

Interference can occur between competing enteroviruses and fecally derived reoviruses (Lieberman 2002) in laboratory BGM cell culture. That is, enteroviruses may be present, but not detectable due to the presence of reovirus (Carducci et al. 2002). In addition, some viruses do not grow well in BGM cells. Other (specialized) continuous cell lines are used to identify more fastidious human viruses (viruses that grow only in certain cultures), such as hepatitis A and rotavirus, which reproduce better (but not well) on Fetal Rhesus Monkey kidney (FRMK) cells or MA-104 cell lines (Gerba 1988). A comprehensive analysis of the various cell lines is documented in Dahling and Wright (1986).

***Detection of Enteric Viruses (Standard Method 9510, APHA 1998):*** This test is entirely based on cell culture assays for detecting viruses in water samples. First, a representative sample of 50-1,500L is collected and concentrated by filtration. The sample concentrate is inoculated onto monolayers of chimpanzee-derived cells (usually BGM cells but other cell lines may be used) in a series of flasks for 2 weeks. Absence-presence is determined based on plaque formation or presence of microscopic cytopathic effects (cell toxicity or death). Positive samples are frozen and thawed, and the burst cell contents are inoculated into a second series of flasks for another 2 weeks; plaque formation or cytopathic effects in this second set confirms the sample was virus-positive. Virus types can then be identified using serological tests. (Concentration results are expressed in three ways: number of PFU; as a most-probable number of infectious units (MPN or MPNIU); or as 50 percent infectious or lethal dose (ID<sub>50</sub> or LD<sub>50</sub>).)

Appendix A describes other viral pathogen monitoring methods currently in development, such as PCR and RT-PCR, used in key occurrences studies documented in Chapter 6.

## **5.5 Conclusions**

The data on available microbial monitoring tools for monitoring fecal contamination suggest that current indicators (*E. coli*, enterococci, coliphage) best meet the following requirements so as to be used as a monitoring tool in the GWR: available, sensitive, precise, accurate, and inexpensive. The costs for typical monitoring methods used in the GWR are described in the *Technology and Cost Document for the Final GWR* (USEPA 2006b).





## 6.0 Occurrence Analysis

### 6.1 Background

To produce a national occurrence estimate of viral and bacterial pathogens and fecal indicator organisms for ground water supplying public water systems (PWSs), EPA has evaluated a number of key studies. Estimates are needed for the population of interest, which is the set of all wells subject to provisions of the GWR. The data from these studies are important to Ground Water Rule (GWR) development because they provide insight on:

- The extent to which ground water may be contaminated by pathogenic viruses
- Possible fecal indicators for source water monitoring under the GWR
- Risk factors, including hydrogeologic sensitivity, that may be used to target monitoring and refine monitoring strategies

Section 6.2 provides brief descriptions of 23 studies on occurrence of pathogens and indicators and their summarized data. Section 6.3 describes the implications of wells located in hydrogeologically sensitive aquifers on contamination levels. Section 6.4 describes modeling and data for co-occurrence of pathogens with indicator bacteria and viruses. Section 6.5 addresses vulnerability of wells, and section 6.6 describes virus concentration data and modeling.

### 6.2 Specific Studies on Occurrence of Pathogens and Indicators

This section provides an updated summary of the studies reviewed by EPA in order to estimate national pathogen and indicator occurrence. EPA reviewed data from 24 recent studies of pathogen and fecal indicator occurrence in ground waters that supply PWSs. EPA selected 15 of these studies to use in evaluating GWR benefits (see the *Economic Analysis for the Final Ground Water Rule* (USEPA 2006a)). Each study was conducted independently and with a unique objective and scope. For example, one data set, Lieberman et al. (2002), targeted wells based on presence of total coliforms and other indicators of vulnerability to fecal contamination. Another data set, Abbaszadegan et al. (2003), targeted a representation of wells throughout the United States based on hydrogeological conditions, but excluded any wells that were poorly constructed or without well logs. Other studies sampled subsets of wells in particular states or in certain hydrogeologic settings within states. Aside from recognizing the numbers of wells surveyed, this analysis makes no attempt to weight any of the studies to compensate for any perceived over- or under-representation of the subset as compared with the total population.

A review of four recent studies of private residential wells is also presented in this section. While residential wells are not regulated under the GWR, these selected studies provide insight on certain aspects of the estimation of occurrence. Residential wells may be constructed to shallower depths because lesser well bore storage is typically adequate, and they may be located closer to potential fecal contamination sources than PWS wells, depending on the date of their construction and state regulations governing private wells. Because they may be shallower and closer to contaminant sources, it is possible that residential wells are at greater risk for fecal contamination than PWS wells. Data from residential wells may be useful to GWR development because they provide additional insight on the extent to which ground water may be contaminated. They also provide information on the co-occurrence of *E. coli* and enteric viruses.

Exhibit 6.1 shows 20 studies of PWSs (and studies of both PWSs and private wells) and Exhibit 6.2 shows the four private well studies. Individual studies may not be representative of national occurrence. The number of samples collected from each well, the assay methods, and the sample volumes, which affect interpretation of results, also varied among the studies. Even within some studies, data may not be representative of local or regional conditions in that the wells were only selected from those systems that volunteered to be included.

Each occurrence study investigated a combination of different pathogenic and indicator viruses and bacteria. The samples analyzed in some studies were tested for enteroviruses, and some also tested for bacterial pathogens such as *Legionella* and *Aeromonas*. Several studies used polymerase chain reaction (PCR) (for which there is no standardized method) to determine the presence of pathogenic viruses.

In the occurrence studies, samples were screened for one or several bacterial indicators of fecal contamination, including enterococci (or fecal streptococci), fecal coliforms (or *E. coli*, which is a fecal coliform), and *Clostridium perfringens*. Samples were also examined for bacteriophage (viruses that infect host bacteria), which are used as viral fecal indicators. Most bacteriophage sampling was targeted on coliphage (i.e., specifically, bacteriophages that specifically infect *E. coli*) identification. Coliphage assays included somatic coliphage and/or male-specific coliphage. *Bacteroides* phage were also investigated in two studies. Most studies also tested for total coliforms, although their presence is not considered a direct fecal indicator.

When evaluating enteroviral occurrence data, it is important to realize some of the fundamental challenges in characterizing enterovirus occurrence; these include detection, identification, and concentration. Key issues include limitations of enterovirus sampling and analytical methods and the difficulties of estimating the probability of virus presence and the associated virus concentration from the measured data.

For analyses for the final GWR, EPA relied only on the identification of enterovirus in PWS wells using cell culture methods, since they allow identification of infectious pathogenic viruses (polymerase chain reaction (PCR) does not distinguish between infectious and non-infectious viruses). However, as further described below, these measurements are likely underestimates of the actual occurrence. Virus recovery can range from less than 20 percent to greater than 50 percent (Dahling 2002; Denis-Mize et al. 2004; Sobsey and Glass 1984).

Viruses often aggregate in water or solution. Methods that count host cell infection cannot differentiate between virus aggregates and solitary viruses and therefore count them all as solitary viruses (Teunis et al. 2005; Young and Sharp 1977). Where multiple virus strains co-occur in plaque assays, statistical analysis has shown that the actual concentration can be as much as 45 percent greater than the concentration determined by the standard method, the plaque assay count (Teunis et al. 2005).

Furthermore, concentration estimates derived from measured values of infectious viruses will be underestimates of the actual concentration because some viruses (such as reovirus) may be favored in the cell line used for testing and may out-compete other viruses (such as echovirus) (Carducci et al. 2002). Among the enteroviruses, slower growing enteroviruses are not favored for recovery and identification.

The number of samples taken at a site and the sensitivity of measurement can significantly influence the observed concentrations. Viral concentrations at one site taken at different points in time can vary, ranging from below detection levels to several orders of magnitude above (Lieberman et al. 2002). This is because the well may experience short-term contamination, or it may have high concentrations for

sustained durations. If a site is only sampled once, it is difficult to say whether the concentration observed at that time is representative of the contamination at the well over the long term.

## Exhibit 6.1 Microbial Pathogen and Indicator Studies/Surveys for PWS Wells

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
1a. EPA/AWWARF Phase I Study (Lieberman et al. 2002)	94 wells; 22 states plus PR and USVI	One sample, 1 L	Somatic coliphage 5/94; 1* Total coliform 31/94; 9* <i>E. coli</i> 19/94; 5* Enterococci 17/94; 3* <i>C. perfringens</i> 4/94; 0* *indicates number of wells positive in Phase I that were not positive or not sampled in Phase II	
1b. EPA/AWWARF– Phase II Study (Lieberman et al. 2002, Fout et al. 2003)	30 wells, of which 23 were from Phase I; 17 states plus PR and USVI	Monthly for 1 year during 1992-94 Average volume filtered: 6,037 L Microscopic Particulate Analysis (MPA) data available for each well; samples frozen for development of a method; 321 samples used in Fout et al. 2003 for molecular analyses (RT-PCR)	Somatic coliphage (16/30) Male-specific coliphage (6/30) <i>Bacteroides</i> bacteriophage (6/30) Total coliform (24/30) Enterococci (21/30) <i>C. perfringens</i> (10/30) <i>E. coli</i> (15/30)	<i>Legionella pneumophila</i> (7/30) Cell Culture: enterovirus (7/30)* PCR: enterovirus (15/321 samples) Hepatitis A virus (4/321 samples) Norwalk (9/321 samples) Reovirus (33/321 samples) Rotavirus (0/321 samples)  *Includes 3 GWUDI sites
2. AWWARF/ AWWSCo Study (Abbaszadegan 1999a,b,c, 2002; Abbaszadegan et al. 2003)	448 wells; 35 states	One sample (25 wells sampled twice); 539 samples total, not all analyses conducted on all samples Sampling volumes: 1512L eluted for virus analyses (5 liter equivalent for RT-PCR, 600L for cell culture), Coliphage 15L, Bacteria 200 mL.	Somatic coliphage, host <i>E. coli</i> C (18/444) <i>E. coli</i> C-3000 host (48/444) Male-sp. coliphage, host Salmonella WG-49 (42/440) Total coliform (44/445) Enterococci (31/355) <i>C. perfringens</i> (1/57) <i>E. coli</i> was not monitored	Cell Culture: enterovirus (21/442)* PCR Rotavirus (62/448) Hepatitis A virus (31/448) Norwalk virus (3/317) Enterovirus (68/448) * one well twice positive

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
3. Pennsylvania Non-Community Wells (Lindsey et al. 2002)	60 wells (15 karst; 25 fractured bedrock; 5 sandstone; 5 sand)	59 samples (not clear which well was excluded) Coliphage sample volume 200-1000 L Bacterial indicators sample volume 100 mL Enteric virus sample 200-1000 L <i>H. pylori</i> 500 mL	Male-specific coliphage (3/59) Somatic coliphage (5/59) Total coliform (27/59) <i>E. coli</i> (8/59) Enterococci (7/59) <i>C. perfringens</i> (9/59)	Cell culture: enterovirus (5/59) <i>H. pylori</i> (by PCR) (4/59)
4. Southeast Michigan (Francy et al. 2004)	38 wells, shallow sand and gravel aquifers	169 regular samples and 32 replicate pairs. 34 wells (93 samples) were analyzed for enteric viruses	Total coliforms (13/38) (15/152 samples) <i>E. coli</i> (4/38) (4/163 samples) enterococci (6/38) (7/158 samples) Male-specific coliphage (2/34) (2/117 samples) Somatic coliphage (1/34) (1/118 samples)	Cell culture: enterovirus (2/34) (2/93 samples) RT-PCR: enterovirus (4/38) HAV (5/38) Rotavirus (0/34) Reovirus (0/34) Norovirus (0/34)
5. New Jersey (Atherholt et al. 2003)	26 wells (12 were GWUDI, 1 well whose source was not identified in the results was not included in the groundwater results)) Unconfined aquifer	128 samples collected between June 1999 - February 2002. Wells sampled 1-10 times each. Bacteria sample volumes were 100 mL. Coliphage sample volumes were 100 mL, but a few were larger.	TC (8/26 total, 3/13 GW) <i>E. coli</i> (3/26 total, 0/13 GW) Enterococci (2/26 total, 1/13 GW) Somatic coliphage (CN 13 host) (5/26 total, 4/13 GW) Male-specific coliphage (Famp host) (5/26 total, 1/13 GW)	
6. Missouri Ozark Plateau Study #1 (Davis and Witt 2000)	109 wells	Two samples/well, 25 wells sampled once for tritium, 200-300 L ground water filtered at the well head	Somatic coliphage (1/109) Male-specific coliphage (10/109) Fecal streptococci (1/109) Fecal coliform (2/109) <i>E. coli</i> (0/109)	Cell Culture: enterovirus (1/109)* PCR: enterovirus (13/109)  *poliovirus overgrowth of cell culture-positive sample (personal communication, Davis and Phil Berger, EPA)
7. Missouri Ozark Plateau Studies #2 (Femmer 2000) (pre-1970 wells)	109 wells	One sample/well, 200-300 L filtered at the well head	Somatic coliphage (3/106) Male-specific coliphage (3/106) Fecal streptococci (8/106) Fecal coliform (8/106) <i>E. coli</i> (9/106)	Cell Culture: enterovirus (0/106)

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
8. Wisconsin Migrant Worker Camp Study	21 wells in sand or sandstone aquifers	Monthly: Bacteria - 6 mos. Phage - 5 mos. Bacteria - 100 mL Phage - 1L	Male-specific coliphage (20/21) Total coliform (14/21) <i>E. coli</i> (0/21) <i>K. pneumoniae</i> (1/21)	
9. New England Study (Doherty et al. 1998)	124 wells primarily in unconfined and bedrock aquifers; 6 states	Each well sampled four times over 1 year  Up to 1500-L sample for virus	Study in progress Male-specific coliphage (4/79) Somatic coliphage (1/70) Total coliform (27/124) <i>Aeromonas hydrophila</i> (19/122) <i>C. perfringens</i> (6/119) <i>E. coli</i> (0/124) Enterococci (20/124)	Cell Culture: enterovirus (0/122) PCR: enterovirus (results not available)
10. Three State Study (Wisconsin, Maryland, Minnesota) (Wisconsin Department of Health 2000; Banks et al. 2001; Banks and Battigelli 2002)	76 from MN, 25 from WI, 27 wells from MD sandy coastal plain, 91 from MD piedmont fractured bedrock.	Each MN well sampled four times over 1 year; each MD well sampled once; each WI well sampled eight times over 2 years Virus sample volume 1500 L Bacteria sample volume 100 mL	Somatic coliphage (1/30 in MD coastal); (2/25 in WI)(4/86 in MD bedrock) Male-specific coliphage (2/76 in MN); (2/30 in MD coastal); (2/25 in WI)(2/88 in MD bedrock) <i>Bacterioides fragilis</i> phage (2/30 in MD coastal)(1/90 in MD bedrock) <i>C. perfringens</i> (0/51 in MN); (0/30 in MD coastal); (0/25 in WI)(1/90 in MD bedrock) Total coliform (12/76 in MN); (4/30 in MD coastal); (11/25 in WI)(19/90 in MD bedrock) Enterococci (1/51 in MN); (2/30 in MD coastal); (1/25 in WI)(9/90 in MD bedrock) <i>E. coli</i> (1/76 in MN); (2/25 in WI)(1/90 in MD bedrock)	Cell Culture: enteric virus (1/25 in WI); (1/27 in MD coastal)*; (0/25 in MN)(0/91 in MD bedrock) RT-PCR: enteric virus (3/30 in MD coastal); (3/25 in WI); (1/25 in MN)(11/91 in MD bedrock)  *positive cell culture in rhabdomyosarcoma (RD) cells was serotyped to be rotavirus using Rotatest in MD coastal; this is a non-standard assay
11. EPA Vulnerability Study (USEPA 1998b)	30 wells; 8 states	Each well visited once  Two 1L grab samples and 1500-L sample Equiv. vol. 650L for enterovirus, 100 mL for bacteria, 10 mL to 100L for coliphage	Male -specific coliphage (0/30), Somatic Coliphage (2/24; large volume) Total coliform (4/30) Enterococci (0/30)	Cell Culture: enterovirus (0/30) PCR: HAV (1/30), rotavirus (0/30), Norwalk (0/30), enterovirus (0/30)

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
12. Montana (Miller and Meek 1996)	38 wells near Helena, Montana (18 PWSs, 20 private) (12 bedrock, 26 valley fill)	Wells sampled 1-3 times	<i>E. coli</i> (0/38) Enterococci (2/38) [uncertain if the positive wells were PWS wells] Male specific coliphage (0/38) Somatic coliphage (0/38)	
13. Karim 2003, 2004	20 wells (California-2, Illinois-2, Indiana-3, Massachusetts-2, Missouri-2, New Hampshire-2, New Jersey-2, New Mexico-1, Ohio-1, Pennsylvania-3)	Each well was sampled monthly for a year.  All indicators sampled using 100 mL and 1L samples (except coliphage Method 1602, which used only 100mL samples)  Coliphage analyzed using Method 1601 and 1602  Enteric virus samples were 1,500 L	Method 1601: Male-specific coliphage (1/20 for 100mL sample, 4/20 for 1L sample) Somatic coliphage (0/20)  Method 1602: Male-specific coliphage (12/20) Somatic coliphage (2/20)  Total coliform (13/20 for 100 mL sample, 16/20 for 1L sample) <i>E. coli</i> (5/20 for 100 mL sample, 7/20 for 1L sample) Enterococci (1/20 for 100 mL sample, 7/20 for 1L sample) <i>C. perfringens</i> (1/20 for 100 mL sample, 3/20 for 1 L sample).	Cell culture: enterovirus (2/20), Rotavirus (5/20), RT-PCR: enterovirus (5/20), rotavirus (9/20), norovirus (8/20), adenovirus (1/20)
14. USEPA 2006c Methods 1601 and 1602 Field Test	SE region (13 in NC and 4 in FL) (8 private)  SW region (TX, NM) -11 wells (all PWSs)  Upper Midwest (MN) -25 wells (6 private)  NE region (12 in NH, 4 in ME, 3 in VT, 6 in MA) - 25 wells (17 private)	Coliphages sampled using Methods 1601 (single agar layer (SAL) (1L)) and 1602 (enrichment (100mL))  Bacteria sampled using EPA-approved methods (100 mL)  Enteric viruses samples using EPA ICR method (1,500 L)  Samples from each region were analyzed by different labs	Somatic coliphage SAL (19/116 samples) (16.4%) F+ coliphage SAL (13/116 samples) (11.2%) Total coliphage SAL (14/116 samples)(12%) Somatic coliphage enrichment (8/116 samples) (6.9%) F+ coliphage enrichment (4/116 samples) (3.4%) Total coliphage enrichment (6/116 samples) (5.2%) Fecal coliform (11/80 samples) (13.8%) <i>E. coli</i> (5/116 samples) (4.3%) Enterococci (14/116 samples) (12.1%)	NE (cell culture/PCR)- astrovirus (0/25) adenovirus (0/25)  SW (cell culture/PCR or RT-PCR) adenovirus (0/27 samples) astrovirus (0/27 samples) enterovirus (0/27 samples) reovirus (0/27 samples) rotavirus (0/27 samples) hepatitis A (0/27 samples) calicivirus (0/27 samples)  Upper Midwest (cell culture/RT-PCR) all samples (including those from other labs) were negative

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
15. Borchardt et al. 2004	6 PWS wells in sand and gravel aquifer near the Mississippi River in La Crosse, Wisconsin	Sampled monthly for one year. Two wells were shut down during one sampling period; samples from nearby wells were used for that period.  2 wells have 12 samples; 2 wells have 11 samples; and 2 wells have 1 sample.	TC (0/6) <i>E.coli</i> (0/6) Enterococci (0/6) Somatic coliphage (0/6) Male-specific coliphage (0/6)	RT-PCR: Enterovirus (5/6) Rotavirus (4/6) Hepatitis A (3/6) Norovirus G1 (3/6) Norovirus G2 (0/6) Cell culture: Enterovirus (0/6) Hepatitis A (3/6)
16. De Borde 1995	2 wells in Missoula, Montana	Sampled monthly for one year.	F+Coliphage (1/2) (8% of samples) Somatic coliphage (0/2)	Enterovirus (0/2)
17. Missouri Alluvial Study	81 wells	Sampling occurred during a 4-month period. Some sampling done during flooding. Some wells sampled more than once.	Somatic coliphage (1/81) Male-specific coliphage (1/81) <i>Bacterioides</i> bacteriophage (1/81) Total coliform (33/81) Fecal coliform (5/81) Fecal streptococci (12/81)	Cell Culture: enterovirus (12/81)
18. U.S.-Mexico Border Study (TX and NM) (Pillai 1997)	17 wells	3 (300-1000 gallon) samples/well	Male-specific coliphage (0/17) Somatic coliphage (0/17)	Cell Culture: enterovirus (0/17)
19. Oahu, Hawaii Study	Virus - 32 wells Bacteria - 39 wells	Each well sampled 1-4 times; total 79 samples Virus – 1-L <i>C. perfringens</i> , H.C.– 0.1L Coliforms, fecal strep – 0.1L and 0.5L	Male-specific coliphage ( 0/32) Somatic coliphage ( 0/32) Total Coliform (3/39) <i>E. coli</i> (1/39) Fecal Streptococci (1/39) <i>C. perfringens</i> (0/39)	<i>Legionella</i> sp. (PCR; 15/26) <i>Legionella pneumophila</i> (PCR; 1/27) None found in Phase 2



## Exhibit 6.2 Microbial Pathogen and Indicator Occurrence Studies/Surveys in Private Domestic Wells

Study	# PWS Wells Sampled & Location	Sampling Frequency/ Volume	Indicators Monitored (# Pos. Wells/# Wells Total, Unless Otherwise Indicated)	Pathogenic Viruses, <i>Legionella</i> (# Pos. Wells/ # Wells Total, Unless Otherwise Indicated)
1. SE Minnesota Residential Wells: Viruses and Drug Resistant Bacteria in Ground Water (Goyal et al. 1989)	24 wells 2 springs  SE Minnesota	268 samples during a 34-month period, 400 L sample	Somatic coliphage (6/17)	Cell Culture: enterovirus (1/24)
2. U.S. Geological Survey (USGS), Regional aquifer study, occurrence and distribution of microbial indicators in ground water and surface water (Francy et al. 2000)	130 wells (includes 24 USGS monitoring wells)	130 samples 100 mL samples	Total coliform (26/130) Fecal coliform (3/130) <i>C. perfringens</i> (0/50)	
3. CDC- Assisted Midwest Well Study (CDC 1998)	5,520 wells	Sampled May-November 1994	Total coliform (2279/5520) Fecal coliform (618/5520)	
4. Water Quality of Domestic Wells in Dona Ana County, New Mexico (Daniel B. Stevens & Associates, Inc. 1996)	135 wells (28 wells sampled for enterovirus by cell culture)	26 wells sampled twice (once for coliphage and again for enteroviruses)  All other wells sampled once	Male-specific coli phage (20/116) Total coliform (22/135) Fecal coliform (4/28) Fecal enterococci (4/28)	Cell Culture: enterovirus (0/28)

## **6.2.1 EPA/American Water Works Association Research Foundation (EPA/AWWARF) Study (Lieberman et al. 2002, Fout et al. 2003)**

### *Study Objectives*

The major objectives of the Lieberman et al. 2002 study were: 1) to obtain occurrence data for infectious human enteric viruses using the BGM cell line, 2) to assess the microbial indicators of fecal contamination, and 3) to develop and to evaluate a molecular biology monitoring method (PCR) to identify viral genomic material without consideration of the infectiousness of that material. The objectives were accomplished by sampling wells to confirm total coliform presence and to establish the presence of other fecal indicators, including somatic coliphage (Phase I) and by choosing a subset of these for monthly sampling for 1 year (Phase II). Wells were nominated for sampling in Phase I by federal, State and local drinking-water experts.

### *Well Selection*

In Phase I, 180 wells were nominated, and 98 were selected. Each selected well was sampled once for total coliform, *E. coli*, enterococci, *Clostridium perfringens* spores, and somatic coliphage. Four additional wells were excluded due to problems with the indicator samples, leaving 94 wells in Phase I. Nominated wells were identified using historical total coliform occurrence data and any other available information about the well, such as confirmed waterborne disease outbreaks, proximity to known sources of human fecal contamination and, in some cases, siting in a sensitive hydrogeologic setting (e.g., karst). Selected wells were located in 22 States, Puerto Rico, and the U.S. Virgin Islands. The wells from Phase I served as the well selection pool for 21 of the 30 wells chosen for Phase II sampling.

Twenty-seven of the 30 wells selected in Phase II had a history of total or fecal coliform occurrence or had any indicator occurrence during Phase I sampling. In aggregate, the 30 wells selected for monthly sampling represent a group of wells considered to be vulnerable to fecal contamination primarily due to historical indicator occurrence, but also due to a positive somatic coliphage, enterococci, or other indicator results from a single sample during Phase I sampling. Proximity to fecal contamination sources, high nitrate concentrations, and location in a sensitive hydrogeologic setting were additional selection criteria for several additional wells. The 30 selected wells, located in 17 States and 2 U.S. territories, were sampled monthly for 1 year for total coliform, *E. coli*, enterococci, *Legionella* species, *Clostridium perfringens* spores, somatic and male-specific coliphage, *Bacteroides* bacteriophage and enteric viruses using BGM cell line.

### *Sample Results*

For viral analyses using cell culture assays, 7 of the 30 wells (23 percent) were positive for enterovirus (3 of the 7 positive wells were designated as ground water under the direct influence of surface water (GWUDI) by the State) and 20 samples (6 percent) were positive for enterovirus or reovirus. While 7 of the 30 wells sampled had a cell culture positive among the twelve samples taken, most of the measurements were below detection levels. One of the wells had 5 monthly viral positives, two of the wells had 4 monthly positives, one of the wells had two monthly positives, and three wells had one positive. Viral strains identified by serotyping included coxsackievirus and echovirus, as well as the enteric virus reovirus. Virus-positive samples ranged in concentration from 0.9–212 PFU or MPN/100 liters with a mean infectious virus concentration of 30.66 PFU or MPN/100 liters (PFU, or plaque forming units, and MPN, or most probable number, are estimates of concentration) among all the positive samples.

RT-PCR (reverse transcription-polymerase chain reaction) analyses were performed on 321 samples from 29 Phase II wells (Fout et al. 2003). When reported as a percentage of wells (not samples), enterovirus-positive results were found in 38 percent, rotavirus-positive in 0 percent, HAV-positive in 14 percent, Norwalk virus-positive in 21 percent, and reovirus-positive in 62 percent of wells. In total, 50 of 321 samples were virus-positive (16 percent). Using cell culture assays, 23 percent of the 30 wells contained enterovirus (20 of 332 samples or 6 percent) (Lieberman et al. 2002). Strains were identified by serotyping. Coxsackievirus and echovirus, as well as reovirus, were identified in the samples. The range in virus concentration in enterovirus-positive samples was 0.9-212 MPN/100 liters with a mean concentration of 28.62 MPN/100 liters (MPN, or most probable number, is an estimate of concentration).

#### *Data Representativeness*

Most of the wells selected as part of Phase II of the Lieberman et al. 2002 study had a history of total coliform occurrence that was confirmed by Phase I sampling. Because most (but not all) of the wells selected for inclusion in the study had a history of fecal contamination, these data are not representative of all PWS wells in the United States, because not all wells in the United States have a history of fecal contamination.

The GWR is concerned primarily with ground water sources vulnerable to contamination, especially the undisinfected sources. Most of the Lieberman et al. 2002 study wells, however, already employ disinfection, which potentially introduces a bias to the data (i.e., the use of disinfection could be considered an indication that the source is known to be contaminated). However, the use of disinfection does not necessarily correlate with known contamination. One enterovirus-contaminated well in the Lieberman et al. 2002 study was undisinfected and had the highest virus concentration for any single monthly sample of the entire study. Another factor that mitigates against this potential bias is that many States and some water systems require ground water disinfection as a matter of policy. For the EPA/AWWARF study, 10 of the 30 wells are located in Alabama, Florida, or Texas; States that require disinfection of all ground water sources. The existence of disinfection at a ground water system may not be directly correlated with indicator occurrence at that facility; therefore, any selection bias is unknown.

### **6.2.2 The American Water Works Association Research Foundation (AWWARF) and American Water Works Service Company (AWWSCo) (AWWARF/AWWSCo) Study (Abbaszadegan et al. 2003 )**

#### *Study Objectives*

Among the objectives of the Abbaszadegan et al. (2003) study were: 1) to determine the occurrence of virus contamination in source water of public ground water systems, 2) to investigate water quality parameters and occurrence of microbial indicators in ground water and possible correlation with human viruses, 3) to develop a statistically based screening method to identify wells at risk of fecal contamination, and 4) to develop and evaluate a molecular biology monitoring method (PCR).

#### *Well Selection*

Wells were selected for the Abbaszadegan et al. (2003) study from a pool of 750 wells. The study was initiated as a study of 150 samples from AWWSCo wells selected to test and evaluate the PCR method (Abbaszadegan et al. 1999a,b,c). With additional funding, the study was expanded to 539 samples from 448 wells in 35 states. The additional wells were nominated by State drinking water program or water utility staff. Study personnel requested nominations of wells not known to be vulnerable to

microbial contamination. Sites selected for that effort were chosen based upon their widely varying characteristics, such as very high or low mineral and metal content, pH, and temperature. The researchers excluded 12 samples included in the first 150 AWWSCo well samples because they were believed to be under the direct influence of surface water and therefore especially vulnerable to contamination. Other nominated wells were excluded if well records were not available or if the well was improperly constructed. All nominated wells were profiled by the well operators or their designees using a questionnaire that included a checklist of 11 different hydrogeologic settings. Results of the study show that approximately 64 percent of the wells are located in unconsolidated aquifers, 27 percent are located in consolidated aquifers, and 9 percent are located in unknown geology. Samples were collected from different geographical locations with a variety of physical and chemical characteristics. This was done to closely match the actual national geologic profile of ground water sources in order for researchers to use the information to generate national occurrence estimates. These percentages are similar to those of national ground water production from unconsolidated and consolidated hydrogeologic settings (modified by Abbaszadegan et al. from USGS circular 1081 1990).

### *Sample Results*

Source water samples were taken from each well and analyzed using a variety of methods to detect pathogens and indicators. Samples were analyzed to determine the occurrence of viruses (using both cell culture and polymerase chain reaction (PCR) methods) and total coliform (TC), enterococci, and *C. perfringens* bacteria in ground waters of the United States. A total of 539 samples were obtained. Not all analyses were conducted on all samples, and 25 wells were sampled two or more times. Information was not available to identify which wells were sampled multiple times. Because the majority were sampled once, and having no other recourse, EPA treated each sample as though it was the only one assayed for a well. PWSs performed the sampling and were given training on procedures to collect at least 400 gallons (1,512 L) of water prior to disinfection. Exhibit 6.3 presents a summary of the Abbaszadegan et al. (2003) study results.

### **Exhibit 6.3 Results of the Abbaszadegan et al. (2003) Study**

<b>Assay</b>	<b>Percent of Sites with Positive Samples (No. positive/samples analyzed)</b>
Enterovirus (cell culture)	4.8%
Total coliform	9.9%
Enterococci	8.7%
<i>Clostridium perfringens</i> spores	1.8%
<i>Male specific coliphage</i> ( <i>Salmonella</i> WG-49 host)	9.5%
<i>Somatic Coliphage</i> ( <i>E. coli</i> C host)	4.1%
<i>Somatic and Male Specific Coliphage</i> ( <i>E. coli</i> C-3000 host)	10.8%
Norwalk virus (RT-PCR)	0.9%
Enterovirus (RT-PCR)	15.2%
Rotavirus (RT-PCR)	13.8%
Hepatitis A Virus (RT-PCR)	6.9%

Source: Abbaszadegan 2002; Abbaszadegan et al. 1999, 2003

## *Data Representativeness*

The Abbaszadegan et al. (2003) study included a large number of wells that were specifically chosen to be representative of the range and proportion of the hydrogeological settings of the United States.

To further evaluate the representativeness of the wells with respect to hydrogeologic conditions, EPA subsequently compared nitrate concentrations from a national database of nitrate concentrations in ground water (Lanfear 1992) with nitrate data measured in the Abbaszadegan et al. (2003) study wells to determine if there was any statistically significant difference between the nitrate levels in the two data sets. Nitrate was chosen for this comparison because a large, national database is available. The national nitrate data were selected randomly from a database of more than 100,000 wells. Using U.S. Census data, EPA stratified the nitrate data into rural and urban components and chose a small random subset of these, comparable in size to the sample in the Abbaszadegan et al. (2003) study (all available Abbaszadegan et al. (2003) study data were used), for comparison. The analysis showed that the Abbaszadegan et al. (2003) study wells had nitrate concentrations that were not significantly different from the national data or from the urban and rural components. Thus, using nitrate concentration as a surrogate, EPA further verified that, by this measure, the Abbaszadegan et al. (2003) study wells data appear to be nationally representative of hydrogeological conditions in the United States.

A potential bias of the Abbaszadegan et al. (2003) study, as with the Lieberman et al. 2002 study, is that the majority of the study wells already employ disinfection (see discussion above for implications of this bias). Again, a mitigating factor is that many States and some water system companies require ground water disinfection as a matter of policy. Fifty-two wells from the Abbaszadegan et al. (2003) study are located in Alabama, Florida, or Texas, States that require disinfection of all ground water sources. In addition, a large number of wells in the study are operated by AWWSCo, which also disinfects as a matter of policy. Therefore, the existence of disinfection at a ground water system may not be directly correlated to issues of contamination at that facility.

Because the description of the hydrogeologic setting was selected by the well operator or designee from a checklist, there are potential uncertainties associated with the hydrogeologic setting data. It is possible that the operator had insufficient data to determine the hydrogeologic setting and was unable to easily consult with a hydrogeologist. No analysis was conducted to determine whether the reported hydrogeologic setting data were correct. It would be expected that viruses would more likely be found in sensitive hydrogeologic settings, as was the case with the Lieberman et al. 2002 data, because the ground water flow within those aquifers is faster and more direct, and there are fewer opportunities for virus concentrations to become attenuated due to interaction with the aquifer solid materials.

Lieberman et al. (2002) found higher virus concentrations and a greater range of concentrations than those measured in the Abbaszadegan et al. (2003) study<sup>1</sup>. The Abbaszadegan et al. (2003) concentrations were uniformly low. Because of variations in well water matrix, source density, proximity to the source and the concentrations in each source, and the virus sampling and analytical process, it is impossible to assess the significance of the differing virus concentrations.

Overall, the magnitude and direction of the biases and uncertainties inherent to the Abbaszadegan et al. (2003) study cannot be definitively quantified.

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<sup>1</sup> Using the cell culture method for enterovirus detection, Abbaszadegan et al. identified only poliovirus from wells (Abbaszadegan et al. 1999), as compared with Lieberman et al. (2002), who identified no poliovirus.

### 6.2.3 Pennsylvania Non-Community Well Study (Lindsey et al. 2002)

#### *Study Objectives*

The purpose of this study was to measure pathogen and indicator occurrence in a random stratified sample of non-community water systems (NCWS) wells in primarily carbonate aquifers and crystalline aquifers, which are hydrogeologically sensitive settings. The U.S. Geological Survey (USGS) analyzed samples from 60 NCWS wells from September to January 2001 to assess the occurrence and distribution of pathogens in ground water used for non-community water supplies and indicator organisms (evaluated as surrogates for those pathogens)(Lindsey et al. 2002).

#### *Well Selection*

USGS personnel, in collaboration with the Pennsylvania Department of Environmental Protection (PaDEP), selected random wells from a targeted population of primarily carbonate and crystalline aquifers. Ten wells were chosen in areas underlain by either siliciclastic bedrock or unconsolidated surficial aquifers. An unconsolidated aquifer is non-sensitive but the siliciclastic aquifer can be either sensitive or non-sensitive depending on whether it is considered to be a sandstone or a quartzite. Aquifer sensitivity is best determined by the State, and EPA cannot make that determination based on the available data.

The vast majority of the sites were transient non-community water system (TNCWS) wells. Only two wells were in non-transient non-community water systems (NTNCWSs). Surrounding land use was included as a criterion for selection; a site was more likely to be selected if potential fecal point sources were located nearby. However, water suppliers with known bacterial contamination problems declined to participate while suppliers with no contamination history were much more willing to participate.

#### *Sample Results*

Of 60 wells initially selected, 59 samples were analyzed for culturable viruses, *Helicobacter pylori* (*H. pylori*), total coliform, *E. coli*, *Clostridium perfringens* (*C. perfringens*), somatic coliphage, male-specific coliphage, and enterococcus.

Culturable viruses were detected in 5 wells, *H. pylori* in 4 wells, *E. coli* in 7 wells, total coliform in 27 wells, *C. perfringens* in 9 wells, somatic coliphage in 5 wells, male-specific coliphage in 2 wells, and enterococci in 8 wells.

Of the 5 wells with detectable culturable viruses, two were near 0.21 PFU per 100 L, while the remaining three ranged from 18 to 56 PFU per 100 L. One of the wells was untreated at the time of sampling.

#### *Data Representativeness*

This data set represents the only randomly sampled human pathogenic virus data from TNCWS wells among the 24 studies considered. As such, it is an important data set for representing the large number of untreated TNCWSs in the United States.

## 6.2.4 Southeast Michigan (Francy et al. 2004)

### *Study Objectives*

The purpose of this study of small (serving fewer than 3,000 people) ground water PWSs was to assess the presence of both viral contamination and microbiological indicators of fecal contamination, relate the co-existence of indicators and enteric viruses, and consider the factors that affect the presence of enteric viruses.

### *Well Selection*

Initially, 160 wells from a previously studied USGS National Water-Quality Assessment Program site were proposed based on nominations from local State and county experts. Wells were nominated if they produced from shallow sand and gravel aquifers, were undisinfected, and did not have well construction flaws. The 38 selected wells were randomly selected from the 160 nominated wells. Well screens are typically shallow, ranging from 50 to 150 feet below ground surface. In some places the aquifer is unconfined, but more often the aquifer is semiconfined or confined by glacial till. Where semiconfined or confined aquifer conditions exist, these wells should theoretically be protected from surficial fecal contamination sources. From July 1999 through July 2001, researchers collected a total of 169 regular samples and 32 replicate pairs in southeastern Michigan from 38 wells in discontinuous sand and gravel aquifers. Not all 38 wells were sampled for all parameters. Only 34 wells (93 samples) were analyzed for enteric virus by cell culture.

### *Sample Results*

Samples were analyzed for total coliform, *E. coli*, enterococci, and coliphage. Coliphages were sampled using two methods, the single agar layer method and the enrichment presence/absence method. Samples were also analyzed for enteric viruses by cell culture and RT-PCR.

Two wells (two samples) were positive for enteric viruses by cell culture. Four wells were positive for enterovirus and five for hepatitis A by PCR. Reovirus, rotavirus, and norovirus were negative by RT-PCR, but quality control problems may have occurred early during the study that affected the accuracy of the results for those viruses. Four wells (four samples) were positive for *E. coli*. Six wells (7 samples) were positive for enterococci. Two wells (two samples) were positive for male-specific coliphage and one well (one sample) was positive for somatic coliphage. All wells sampled are undisinfected, so it appears that the semi-confining or confining layers are not sufficient protection against fecal contamination.

### *Data Representativeness*

This study is unique among the 24 studies considered in that it sampled only undisinfected wells. Other studies were typically not able to sample undisinfected wells because well operators did not allow sampling. Thus, this study is representative of the large number of small, undisinfected PWS wells in the United States. Despite the apparent random well selection process, seven wells were not further considered for sampling at the request of the well owner or because they were found to be unsuitable.

## **6.2.5 New Jersey (Atherholt et al. 2003)**

### *Study Objectives*

This study was designed to sample wells in New Jersey for fecal indicator organisms. No samples were analyzed for enteroviruses or other viruses pathogenic to humans. Thus, data from this study was used only to determine the probability that a sample was fecally contaminated by *E. coli*.

### *Well Selection*

Twenty-six PWS wells were sampled for a variety of fecal indicator organisms. Twelve wells were identified as GWUDI and so data from these wells are not used in this analysis. Eighty-one samples were collected from the 13 ground water wells (all in community water systems) (128 samples were collected from all wells) between June 1999 and February 2002. One well with one sample was not reported as groundwater or GWUDI so this value was not included. All of the wells were located in unconfined aquifers. Although GWUDI wells were selected to increase the likelihood that fecal indicator organisms were present, no information is given for the selection of the other wells.

### *Sample Results*

All 13 wells (81 samples) were negative for *E. coli*.

### *Data Representativeness*

These data represent a subset of community ground water wells in New Jersey that produces water from unconfined aquifers.

## **6.2.6 Missouri Ozark Aquifer Study #1 (Davis and Witt 2000)**

### *Study Objectives*

The purpose of this study was to determine the water quality in recently constructed community public water system wells in the Ozark Plateau region of Missouri. This largely rural region is characterized by carbonate aquifers, both confined and unconfined, with numerous karst features throughout. A confining layer is defined in this study as a layer of material that is not very permeable to ground water flow and that overlays an aquifer and acts to prevent water movement into the aquifer.

### *Well Selection*

The USGS, working with the Missouri Department of Natural Resources, selected a total of 109 wells, in both unconfined and confined aquifers (Davis and Witt 2000). In order to eliminate poorly constructed wells from the study, wells that had been constructed within the last 15 years were selected primarily. Wells were also selected to obtain good coverage of the aquifer and to reflect land use (primarily agriculture and forest) and potential sources of contamination (e.g., confined animal feeding operations, onsite wastewater systems). All wells were sampled twice, once during the wet season (May-June) of 1997 and once during the dry season (November-March) of 1998.



### *Sample Results*

Wells were sampled for fecal coliform, fecal streptococci, *E. coli*, coliphages, and human enteric viruses (by cell culture and integrated cell culture-PCR). In addition, a subset of wells considered at risk for contamination from confined animal feeding operations were analyzed for porcine and bovine enteric viruses by cell culture; all were negative. One sample was reported as human enteric virus-positive (non-poliovirus), but this virus-positive well was not used in the data analysis for the GWR because this sample (and others) had some quality assurance problems due to cross contamination of samples with the poliovirus control (personal communication, Jerry Davis and Phil Berger, EPA). Thirteen wells were positive for enteric viruses by PCR. Coliphages were detected in 14 wells, fecal coliform were found in 2 wells, and fecal streptococci were found in 9 wells.

### *Data Representativeness*

These data are representative of wells in the Ozark Plateau aquifer of Missouri. These data potentially underestimate the probability of wells and samples being positive for enteroviruses because one positive well was not included in the data set for the GWR.

## **6.2.7 Missouri Ozark Aquifer Study #2 (Femmer 2000)**

### *Study Objectives*

The purpose of this study is to determine the water quality in older (pre-1970) CWS wells in the Ozark Plateau region of Missouri to supplement Missouri Ozark Aquifer Study #1 (Davis and Witt 1998, 1999, 2000). This largely rural region is characterized by carbonate aquifers, both confined and unconfined, with numerous karst features throughout.

### *Well Selection*

The USGS, working with the Missouri Department of Natural Resources, sampled a total of 109 wells (Femmer 2000), in both unconfined and confined aquifers. Wells (all of which were constructed before 1970) were selected for monitoring to obtain good coverage of the aquifer, and to reflect land use (primarily agriculture and forest). Priority was given to wells that had completion records, well operation and maintenance history, and that were currently being used. Thirty-eight percent of the wells had disinfection. Each well was sampled once (during the spring).

### *Sample Results*

Wells were sampled for fecal coliform, fecal streptococci, *E. coli*, coliphages, and enteric viruses (by cell culture). Less than 10 percent of wells tested positive for bacterial contamination (9 wells were positive for *E. coli*, 8 were positive for fecal coliform, and 7 were positive for fecal streptococci), and 3.7 percent were positive for coliphages. Most of the bacteria-positive samples were from the unconfined aquifer. No wells were enterovirus-positive by cell culture.

### *Data Representativeness*

These data are representative of PWS wells in the Ozark Plateau aquifer of Missouri.

## **6.2.8 Wisconsin Migrant Worker Camp Study (USEPA et al. 1998a)**

### *Study Objectives*

The purpose of this study was to determine the quality of drinking water in the 21 ground water PWSs serving migrant worker camps in Wisconsin (US EPA 1998a). Each well was sampled monthly for 6 months, from May through November, 1997. The study conducted sampling for male-specific coliphage, total coliforms, and *E. coli*. When detection of coliforms occurred, the specific type of coliform was further identified (speciated). One total coliform positive sample was identified to contain *Klebsiella pneumoniae*, which can be due to fecal or non-fecal origins. Along with the microbial indicators, nitrate and pesticides were also measured.

Other factors were compared to the microbial and chemical sampling results of the study. Well construction records were available for 14 of the wells. The mean casing depth was 109 feet (range 40 to 282 feet) and the mean total well depth was 155 feet (range 44 to 414 feet). Most of these 14 wells are also reported to terminate in a sand or sandstone formation.

### *Well Selection*

These TNCWSs are located in three geographic locations across the State.

### *Sample Results*

Investigators detected male-specific coliphage in 20 of 21 wells during the 6 month sampling period but never detected *E. coli*. In addition, four wells had nitrate levels that exceeded the EPA MCL for nitrate. No wells were analyzed for enteric virus by cell culture.

### *Data Representativeness*

The data from this study are intended to be representative only of TNCWSs in migrant labor camps.

## **6.2.9 New England Study (Doherty et al. 1998)**

### *Study Objectives*

The purpose of this study was: (1) to determine the prevalence of enteric pathogens in New England's PWS wells, (2) to assess the vulnerability of different systems, and (3) to evaluate various fecal indicators.

### *Well Selection*

Wells were selected based on the following criteria: (1) must have constant withdrawal throughout the year, (2) must be near septic systems, (3) should have, if possible, a history of violations of the MCL for total coliforms or elevated nitrate levels, and (4) must not have direct infiltration by surface water (Doherty 1998).

Wells were nominated, characterized, selected, and sampled by regulatory staff of Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont. The selection process considered

wells in different hydrogeologic settings. Of the 124 total wells, 69 (56 percent) were located in unconfined aquifers, 31 (25 percent) were located in bedrock aquifers, 10 (8 percent) were located in confined aquifer hydrogeologic settings, and 14 (11 percent) were located in unknown aquifer settings. Each well was sampled quarterly for 1 year. Enterococci were identified in 20 of 124 wells (16 percent) and in 6 of 31 (19 percent) bedrock aquifer wells.

#### *Sample Results*

No wells were positive for enteric virus by cell culture. No wells were positive for *E. coli*.

#### *Data Representativeness*

These wells are intended to be representative of New England PWS wells. Two wells were provisionally identified as cell culture positive (and reported as positive in EPA 2000) but were found to be contaminated in the laboratory when the samples and lab controls were sequenced by CDC.

### **6.2.10 Three State PWS Study (Wisconsin) (Wisconsin Department of Health 2000)**

#### *Study Objectives*

The purpose of the three-state study is to characterize the extent of viral contamination in PWS wells by testing wells in differing hydrogeologic regions and considering contamination over time (Wisconsin Department of Health 2000). The Wisconsin study sampled 25 wells quarterly for 2 years.

#### *Well Selection*

No explanation is available on the method used in selecting the wells.

#### *Sample Results*

One well in Wisconsin was positive for enteric viruses by cell culture.

#### *Data Representativeness*

No information is available to evaluate the representativeness of these data.

### **6.2.11 Three State PWS Study (Maryland) (Banks et al. 2001)**

#### *Study Objectives*

The purpose of this study was to sample shallow wells in Worcester and Wicomico Counties on Maryland's Eastern shore in order to characterize microbiological occurrence and possible factors affecting well vulnerability. Each well sampled was analyzed for enteric viruses by Buffalo Green Monkey (BGM) and RD (human embryonal rhabdomyosarcoma) cell culture, *Bacteroides fragilis*, somatic and male-specific coliphage, enterococci, total coliform, *E. coli*, and *Clostridium perfringens*.

#### *Well Selection*

The selected wells were chosen from 278 small PWS wells by a non-weighted ranking system based on vulnerability. Ranking criteria included factors such as historical fecal coliform occurrence, land

use, well depth and age, and other factors. Twenty-seven wells located in two counties that are underlain entirely by sandy coastal plain aquifers were selected and sampled once between March 1999 and October 1999. Of the 27 samples collected, 11 were from the most vulnerable sites, 13 were from intermediately vulnerable sites, and 3 were from the least vulnerable sites. Three negative control samples were collected and analyzed for the suite of enteric pathogens as well. Two of these control samples were drawn from deep (575 to 600 feet) wells located outside of the study area. A double-filtered sample was collected as the third control.

### *Sample Results*

Analysis showed that 1 well was positive (by RD cell culture) for enteric virus contamination. Fifteen percent of the samples were positive for fecal-indicator bacteria. *B. fragilis* was detected at two sites. Somatic coliphage was detected at one site, and two sites were positive for male-specific coliphage. Two samples had detections of enterococci. Total coliforms were found in 4 samples. Serological testing on virus-positive RD cell cultures in one well confirmed the presence of rotavirus using Rotatest (personal communication, David Battigelli and Phil Berger, EPA). Two other samples from the same well were positive for enteric virus using PCR. None of the wells was positive for *E. coli* or *C. perfringens*.

### *Data Representativeness*

These wells are representative of shallow wells with varying amounts of vulnerability in a sand coastal plain aquifer.

## **6.2.12 Three State PWS Study (Maryland) (Banks and Battigelli 2002)**

### *Study Objectives*

The purpose of this study was to sample shallow wells in the Maryland Piedmont physiographic province. Each well was sampled for enteric viruses by BGM cell culture, *Bacteroides fragilis*, somatic and male-specific coliphage, *E. coli*, *Clostridium perfringens* and TC. One-hundred-one samples were collected from April 10, 2000, to November 13, 2000. This total included ten replicate samples for QA purposes.

### *Well Selection*

For this study, 91 small PWS wells were selected for sampling from 263 wells in the fractured bedrock aquifer of two Maryland Piedmont physiographic province counties. Wells were selected to distribute the sample sites evenly over the population and spatial extent of the study area. One well was selected randomly.

### *Sample Results*

None of the wells was positive for enteroviruses by cell culture. One well was positive for rotavirus by RT-PCR. Seven percent of samples were positive for coliphage or *Bacteroides fragilis* phage. Only one well was positive for *E. coli*, but 26 of 90 samples contained one or more fecal indicator bacteria (enterococci, *C. perfringens*, or *E. coli*).

### *Data Representativeness*

These data are representative of shallow wells in fractured bedrock (sensitive) aquifers with thick soil and weathered rock (saprolite) cover that acts to protect against fecal contamination. This same aquifer setting would be more likely to be contaminated if located further north, where glacial advances during the Ice Age removed much of the weathered soil and rock.

## **6.2.13 Three State PWS Study (Minnesota) (Minnesota Department of Health 2000)**

### *Study Objectives*

The Minnesota study (Minnesota Department of Health 2000) sampled 76 wells. Seventy-four wells were sampled for at least four consecutive calendar quarters. The remaining two wells were sampled for two consecutive quarters each. In addition to microbial indicator and virus data, one sample from each well was also analyzed for tritium and tritium/3helium.

### *Well Selection*

Sampled wells were more likely to be selected if they were small, transient PWSs, and/or were located in a aquifer that was perceived to be vulnerable. Of the 76 Minnesota wells sampled, six (8 percent) served community water systems (CWSs), 19 (25 percent) served NTNCWSs and 51 (67 percent) served TNCWSs. The aquifer types that are utilized by these wells include dolomite (6 wells), dolomite and sandstone (3 wells), fractured crystalline bedrock (9 wells), sandstone (28 wells), sand and gravel (29 wells) and regolith (surficial materials) (1 well).

### *Sample Results*

No wells in Minnesota were enteric virus-positive by cell culture.

### *Data Representativeness*

These data are representative of PWSs in predominantly vulnerable aquifers in Minnesota.

## **6.2.14 EPA Vulnerability Study (USEPA 1998b)**

### *Study Objectives*

The purpose of this study was to conduct a pilot test of a new vulnerability assessment method by determining whether it could predict microbial monitoring results (USEPA 1998b). The vulnerability assessment assigned low or high vulnerability to wells according to their hydrogeologic settings, well construction and age, and distances from contaminant sources.

Samples were taken and tested for enteroviruses (both by cell culture and PCR), hepatitis A virus (HAV) (by PCR), rotavirus (by PCR), Norwalk virus (by PCR), and several indicators (total coliforms, enterococci, male-specific coliphage, and somatic coliphage).

### *Well Selection*

A total of 30 wells in eight States were selected to represent ten hydrogeologic settings. Selection was based on the following criteria: (1) wells representing a variety of conditions relevant to the vulnerability predictions, (2) wells with nearby sources of potential fecal contamination, and (3) wells with sufficient well and hydrogeologic information available.

### *Sample Results*

No wells were positive for enteric virus by cell culture. No *E. coli* data were collected. The only positive result was one PCR sample positive for HAV.

### *Data Representativeness*

Wells were selected to be representative of a variety of hydrogeologic settings in the United States. However, the small number of wells in the study and the large number of hydrogeologic settings makes such a comparison difficult.

## **6.2.15 Montana (Miller and Meek 1996)**

### *Study Objectives*

To sample source water for *E. coli*, enterococci, male-specific and somatic coliphage from wells representing primary aquifer types, bedrock, and valley-fill aquifers.

### *Well Selection*

The water-yielding zones of the 6,000 foot-deep valley-fill aquifer are hydraulically interconnected; therefore, these deposits function as one complex aquifer system. Eighteen small PWS wells (and 20 residential wells) near Helena, Montana, ranging from 39-425 feet deep, were sampled for total coliform, *E. coli*, enterococci, male-specific and somatic coliphages. Twelve wells were in bedrock and the rest were in valley fill. EPA method 1601 (which was proposed but not approved at the time) was used in the collection and analysis of male-specific and somatic coliphage samples. Wells were sampled 3 times (April, June, and November 1995).

### *Sample Results*

No *E. coli*, male-specific or somatic coliphage were detected in any well. Total coliform was detected at 8 sites. Enterococci were detected in two wells but it is not known whether these wells were PWSs or residential. Coliphage results are difficult to reproduce in the field, casting the utility of coliphage as an indicator organism into question. It appears that of the 5 microorganisms, total coliform is the most reliable indicator of contamination.

### *Data Representativeness*

These data are representative of bedrock and valley-fill aquifers.

## 6.2.16 Karim et al. 2003, 2004

### *Study Objective*

The objective of this study was to monitor the occurrence of enteric viruses in the continental United States and to develop a useful microbial indicator for assessing the vulnerability of ground water at risk for fecal contamination.

### *Well Selection*

Twenty wells were selected from a national study of 448 sites from Abbaszadegan et al. (2003). Fifteen of the wells were chosen based on previous enteric virus detections or fecal indicator results. All wells were monitored monthly for one year, with a total of 235 samples collected; they represented a wide range of hydrogeologic settings from 11 states, including consolidated and unconsolidated sedimentary formations with alluvial, sandstone, limestone, and glacial outwash deposits. A second study on the same set of wells was conducted (Karim et al. 2004); however, sampling parameters and the total number of samples for this study are not available.

### *Sample Results*

All 20 sites were positive for at least one of the indicators tested in the first study (Karim et al. 2003, 2004). Two wells were enterovirus-positive by cell culture using BGM cells. Five wells were rotavirus positive using MA-104 cells. Fifteen wells were virus-positive by RT-PCR. Twelve wells were positive for either male-specific or somatic coliphage, 16 wells were positive for total coliforms, 7 were positive for *E. coli* and enterococci, and 3 were positive for *Clostridium*. There appeared to be a seasonal trend in virus occurrence, with summer and early winter yielding more positive samples.

### *Data Representativeness*

These data are representative of vulnerable wells of varying hydrogeology. However, with the available data provided by the researchers (raw spreadsheet data and summary reports), it is impossible to combine the two data sets because the well site identifying characters differ in the two studies. Thus, there is no alternative other than treating the two studies as if they are separate, independent data sets. If treated as two data sets, significant bias is introduced. First, the same well is counted twice. Second, well data are treated as if they are unbiased, independent data, when they actually were selected based on prior sampling.

## 6.2.17 Method 1601 and 1602 Field Testing (USEPA 2006c)

### *Study Objective*

This study was designed to field test new coliphage assay methods. The study also aimed to determine the ability of coliphage indicators to predict the presence of human enteric viruses.

### *Well Selection*

Because the objective was to better identify and count fecal indicators, PWS wells with known fecal contamination were more likely to be selected. However, most of the wells sampled in this study were private wells.

Wells from four regions of the United States were selected and analyzed by four different laboratories. The Southeast wells, analyzed at the University of North Carolina, included 13 wells (2 CWSs, 3 NCWSs, and 8 private) in North Carolina and 4 CWSs in Florida. Wells in the Southwest included 3 PWSs in karst aquifers near San Antonio and 8 in vulnerable aquifers in southern New Mexico. In New England, 8 PWSs and 17 private wells were included. Upper Midwest wells include 6 private wells and 19 NCWSs. Each regional laboratory took 27 samples from its set of wells, except that only 25 samples were taken in New England.

Coliphages were analyzed using both EPA Method 1601 (single layer agar) (USEPA 2001c) and Method 1602 (enrichment) (USEPA 2001d). Enteric viruses were analyzed by cell culture-PCR (or cell culture-RT-PCR), or by RT-PCR if non-culturable.

### *Sample Results*

Somatic coliphage were detected in 19 of 116 samples with Method 1601 and 8 of 116 samples with 1602. Male-specific coliphage were found in 13 of 116 samples with 1601 and 4 of 116 samples using Method 1602. Fecal coliform were detected in 11 of 80 samples, and *E. coli* in 5 of 116 samples. Lastly, enterococci were detected in 14 of 116 samples.

Cell culture-(RT)-PCR for adenoviruses, astroviruses, enteroviruses, hepatitis A, reoviruses, and rotaviruses was negative. RT-PCR (without cell culture) for noroviruses was also negative.

### *Data Representativeness*

The raw data were not provided by the investigators, so counting and analysis of the PWS wells is subject to error. Because many of the wells were not PWS wells and likely represented a biased set of PWS wells, these data are not included in the exposure compilation in the GWR EA.

## **6.2.18 La Crosse, Wisconsin (Borchardt et al. 2004)**

### *Study Objectives*

The primary purpose of this study was to link surface water infiltration (from the Mississippi River) into La Crosse, Wisconsin, municipal drinking water wells with human enteric virus detection. The secondary goal of the study was to link the frequency of enteric virus occurrence to the microbial indicators of water quality.

### *Well Selection*

Four drinking water wells and one surface water site were selected from an alluvial sand-gravel aquifer for their tendency to allow fecal viruses to transport readily to the capture zone of the wells. All wells were properly constructed and all samples were collected prior to chlorination. Through previous hydrogeologic modeling, one well was predicted to have high levels of surface water infiltration, one was predicted to have low levels, and two wells were predicted to have intermediate levels of infiltration. The surface water was collected from a levee within city limits. The samples were taken on a monthly basis for one year (two additional wells were sampled for one month each while two of the regular wells were taken out of service). As a control, two piezometers were installed, and samples were collected half way through the study.



## *Sample Results*

No enteroviruses were detected by cell culture, but HAV virus was detected by cell culture in 3 wells. Fifty percent (24 of 48) of well water samples were positive for at least 1 virus group as detected by RT-PCR. Eleven of 48 (23 percent) were positive for 2 or more virus groups. Viruses that were detected included enteroviruses (20 samples, or 42 percent), rotaviruses (10 samples, or 21 percent), HAV (4 samples, or 8 percent), and norovirus genogroup 1 (3 samples, or 6 percent). Viruses were not detected in one well, well 13, but it was only sampled once. Only wells 10 and 24 contained appreciable amounts of surface water, based on ratios of oxygen and hydrogen isotopes in the water molecules. The relationship between virus occurrence and the amount of surface water infiltrating a well could not be determined because nearly all of the wells were virus positive. Of the 48 samples, 24 were positive by RT-PCR for the same group of viruses (5 of the 6 wells were positive). Norovirus genogroup 2 was not detected in any of the samples. Borchardt et al. (2004) also analyzed total coliform and *E. coli*, as well as somatic and male-specific coliphages (using EPA method 1602). None of these microbial indicators was detected in well samples.

## *Data Representativeness*

The results of this study could be transferable to other communities with similar hydrogeologic characteristics, thus enabling the identification of wells vulnerable to virus contamination without costly sampling of all wells in a pumping system. The relationship being investigated in the primary study would be useful for predicting those wells most vulnerable to virus contamination, indicating where additional treatment might be necessary to produce safe drinking water. Nearly one-third of the ground water withdrawn in the United States is pumped from aquifers similar to those in La Crosse; therefore, the results of this study could be used by a variety of municipalities.

### **6.2.19 De Borde 1995**

This study sampled two wells from the same community monthly from one year. Because this study was small and both wells are located in the same community, these data are not included in the exposure compilation in the GWR EA. Male-specific coliphage was detected in one sample in one well. Somatic coliphage was not detected; nor was enterovirus.

### **6.2.20 Missouri Alluvial Aquifer Study (Vaughn 1996)**

#### *Study Objectives*

The purpose of this study was to determine water quality in wells in areas that were subjected to recent flooding (Vaughn 1996; Duzan 2002 personal communication).

#### *Well Selection*

The wells are located primarily in the thick, wide alluvium of the Missouri and Mississippi Rivers. Sampling occurred during March through June 1996, and most wells were sampled once. A total of 81 wells were sampled and 117 samples were collected, 105 of which were from wells in alluvial aquifers. Twelve additional samples were collected from nine “upland” wells. Of these, six PWS wells were “control” wells and were sited in “deep rock” aquifers.

Fifty-five of the wells are reported to be “flood-affected.” Seventy-five wells were affected by a flood in 1993 and many were affected by a flood in 1995. In addition, some of the wells sampled had been flooded around the surface well casing prior to the sampling event, and “several” were flooded at the time of sampling.

#### *Sample Results*

Twelve wells were enterovirus-positive. Six of the 12 enterovirus-positive wells were reported to be flood-affected at the time of sampling.

#### *Data Representativeness*

Like the wells in La Crosse, Wisconsin, these data represent wells in vulnerable alluvial aquifers. However, because flooding was in progress or recent at the time of sampling, the data may not be representative of normal conditions.

### **6.2.21 US-Mexico Border Study (Pillai 1997)**

#### *Study Objectives*

The purpose of this study was to determine water quality in wells sited in alluvium along the Rio Grande River between El Paso, Texas, and the New Mexico border.

#### *Well Selection*

The 17 wells selected were perceived to be the most vulnerable, based on well depth, chloride concentration, and proximity to contamination sources, especially the Rio Grande River.

The wells tested are relatively shallow and all serve fewer than 10,000 people. One well serves 8,000 people, while seven wells serve fewer than 100 people. Well depths range from 65 feet to 261 feet, but most are about 150 feet deep. This signifies that water was collected from the middle aquifer, a shallow but potable aquifer, as the wells shallower than 65 feet contain chloride concentrations prohibitively high for drinking water. Each well was sampled twice for enteroviruses during the same visit. Samples collected from each well were tested for enteroviruses (by cell culture), somatic coliphage, and male-specific coliphage.

#### *Sample Results*

None of the sites were positive for any of the viruses tested (Pillai 1997).

#### *Data Representativeness*

These data are representative of vulnerable alluvial aquifers.

### **6.2.22 Oahu, Hawaii Study (Fujioka and Yoneyama 2001)**

#### *Study Objective*

The purpose of this study was to establish a water quality monitoring program to assess the microbial quality of deep ground water used to supply Honolulu.

### *Well Selection*

The total number of wells sampled was 71, 32 of which were sampled for viruses and 39 of which were sampled for bacteria. The wells are located in carbonate or basalt aquifers.

Each of the wells was tested for several pathogens and indicators of fecal contamination. Bacterial samples taken from 39 wells (79 samples) were tested for TCs, fecal streptococci, *Clostridium perfringens*, heterotrophic bacteria (by m-H.C.), and *Legionella* (by PCR). Samples volumes were 100 mL for *C. perfringens* and heterotrophic bacteria, and both 100 mL and 500 mL for coliforms and fecal streptococci. For F+RNA coliphage (male-specific RNA coliphage), one liter samples from 32 wells (35 samples) were tested by membrane adsorption-elution method, while 24 wells (24 samples) were tested by an enrichment technique developed by Yanko (Fujioka and Yoneyama 1997, 2001).

### *Sample Results*

None of the wells was coliphage-positive, and one sample each was positive for *E. coli* and fecal streptococci (Fujioka and Yoneyama 2001).

Because fecal microbial indicators are not absolutely reliable surrogates for the fate and movement of human enteric viruses through ground water and soil matrices, Fujioka et al. (1999) conducted Phase 2 of the Oahu study to analyze ground water samples from deep wells using the cell culture method and also by the cell culture-polymerase chain reaction (ICC-PCR) method. With both of these methods, all 45 ground water samples were negative for enteric viruses representing all seven aquifers on Oahu. Because the sensitivity of the ICC-PCR was not sufficient in the laboratory that was used, additional tests on 45 samples were conducted using a modified ICC-PCR method that achieved a sufficient level of sensitivity. All were negative for enteric viruses (Fujioka et al. 1999).

### *Data Representativeness*

The data are representative of vulnerable wells in Hawaii.

## **6.2.23 Southeastern Minnesota Residential Wells (Goyal et al. 1989)**

### *Study Objective*

This study was conducted to determine the presence of human pathogenic enteric viruses, TCs, fecal coliforms, and somatic coliphage in private rural wells of southeastern Minnesota (Olmstead County) (Goyal et al. 1989).

### *Well Selection*

Private wells were selected if their owners had previously requested that the county health department test their wells for nitrate and coliform contamination. Most wells were located within karst hydrogeology. In this study, 268 samples (400 liters in volume) were taken from 24 wells and two springs during a 34-month period.

### *Sample Results*

A positive cell culture for enteroviruses was reported for one well. Somatic coliphage was found in six of 17 wells.

### *Data Representativeness*

Because these data are from private wells, they are not representative of wells subject to the GWR. However, because they are located in karst, in which contaminants may travel long distances, these wells may be subject to the same types of contamination as PWSs located nearby.

## **6.2.24 USGS Regional Aquifer Study (Francy et al. 2000)**

### *Study Objective*

The USGS Regional Aquifer Study collected data on occurrence and distribution of fecal indicators to aid the EPA in developing the drinking water regulations (Francy et al. 2000).

### *Well Selection*

A total of 141 wells were selected for sampling bacterial indicators, including 25 USGS monitoring wells from which 130 samples were collected for a total of 143 ground water samples from all wells.

### *Sample Results*

TCs were found in 20 percent, *E. coli* in less than 1 percent, and *C. perfringens* in none of the ground water samples analyzed. Bacteriophage were not quantified due to laboratory problems. A greater percentage of TC detections (22.5 percent) was found in wells located on properties with septic systems. Some of these wells were GWUDI.

### *Data Representativeness*

These data are representative of vulnerable private wells.

## **6.2.25 Centers for Disease Control (CDC) Assisted Midwest Well Study (CDC Center for Environmental Health 1998)**

### *Study Objective*

The objective of the CDC Assisted Midwest Well Study was to assess the presence of bacteria and chemicals in water drawn from domestic wells in states severely affected by floods.

### *Well Selection*

The 5,520 private wells sampled were distributed through the Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, South Dakota, North Dakota, and Wisconsin areas. The sampling was conducted over the 7 month period between May and November 1994. Well selection was limited to areas severely affected by floods.

### *Sample Results*

TCs were reported in 41 percent (2279/5520) of the wells sampled, with *E. coli* present in 11 percent (618/5520) of the wells (CDC 1998a).

### *Data Representativeness*

Due to the large sample size, these data well represent vulnerable private wells.

#### **6.2.26 New Mexico Border Health Office—Water Quality of Domestic Wells in Dona Ana County (Daniel B. Stephens & Associates, Inc. 1996)**

##### *Study Objective*

The New Mexico Border Health Office–Water Quality of Domestic Wells in Dona Ana County study assessed the quality of water obtained from domestic wells in the New Mexico Mesilla Basin region and characterized the quality of the water consumed by residents in the area.

##### *Well Selection*

The 135 wells were selected based on known or suspected ground water contamination with shallow depth to ground water. At least one sample per well was collected during the winter and spring (February through May) of 1996 (Daniel B. Stephens & Associates, Inc. 1996). The hydrogeologic conditions consist primarily of an alluvial valley with a 4,000-foot thick layer of sediments, bounded on both sides of the Rio Grande by faults that have juxtaposed less permeable volcanic rocks in contact with the alluvial sediments. Twenty-eight wells were sampled and analyzed for enteric viruses by cell culture.

##### *Sample Results*

Cell culture analysis results showed that none of the 28 wells was enterovirus-positive.

### *Data Representativeness*

These data are representative of private wells in alluvial aquifers.

## **6.3 Hydrogeologically Sensitive Wells**

This section describes how sensitive and non-sensitive wells may differ. EPA believes that hydrogeological sensitivity of aquifers is an important factor influencing the hit rates (percentages of wells and samples that are positive for viruses or indicators) and concentrations of contaminants in source water. However, most surveyed wells described in section 6.2 were not identified according to their hydrogeologic sensitivity. The *Economic Analysis for the Final Ground Water Rule* (USEPA 2006a) assumes no difference in viral hit rates or concentrations (when virus is present) in sensitive versus non-sensitive wells, because of insufficient data to make such distinctions.

The virus occurrence analyses described in this chapter combine data from studies with differing study designs. One large study (Abbaszadegan et al. 2003) used a checklist provided to the well operator to identify one of ten hydrogeologic settings. No information is available to determine whether the operator had adequate geologic information, geologic training, or assistance from a hydrogeologist to identify the hydrogeologic setting. The hydrogeologic settings listed in the checklist are not directly identifiable as sensitive or non-sensitive aquifers but require additional interpretation by a hydrogeologist. For these reasons, the hydrogeologic setting data from this study and studies with similar design are not adequate to determine whether a well is located in a sensitive hydrogeologic setting. A few small studies, most designed and conducted by the U.S. Geological Survey (USGS), are suitable for determining whether a well is located in a hydrogeologically sensitive setting. EPA concluded that most data available

to classify wells as located in a sensitive or non-sensitive aquifer were not obtained by the USGS or a trained hydrogeologist.

Based on the Abbaszadegan et al. (2003 study), EPA estimates that 15 percent of wells (and/or entry points, which are used to represent wells in the economic analysis) are located in sensitive hydrogeology. Using the hydrogeological setting information reported in Abbaszadegan et al., EPA estimated that at least 68 of the 448 sites were located in either carbonate, limestone, plutonic igneous and metamorphic, or volcanic aquifers. There is considerable uncertainty in this estimate because the hydrogeologic setting for each well was determined by the well operator using a checklist of hydrogeologic settings. As mentioned above, although the operator may have had geologic data or the services of a hydrogeologist to identify the hydrogeologic setting, it is also possible that the setting was determined based on inadequate information. (See Appendix K of the economic analysis for further description of how sensitivity is used to evaluate impacts of assessment monitoring).

EPA believes that wells located in sensitive aquifers are more likely to be virus- contaminated and, if contaminated, likely to have higher virus concentrations than wells located in non-sensitive aquifers. EPA bases this belief on tracer test studies of ground water flow velocity in sensitive versus non-sensitive aquifers. For example, Renken et al. (2005) determined that dye traveled 100 m in six hours (the first dye arrived in 4 hours) to a production well in a karst (sensitive) aquifer. Travel in non-sensitive aquifers is typically measured to be about a meter per day. Because ground water velocity is so fast, viruses can travel from a source to a well with little time for virus die-off. Furthermore, the open porosity and connected permeability of sensitive aquifers allows virus to travel very efficiently with few opportunities to be delayed by attachment to the aquifer solid materials. EPA believes that a tracer test study that released viruses into the subsurface (like the discharge of septage) at the same location in relation to a well in a sensitive aquifer versus a non-sensitive aquifer would demonstrate the following: 1) viruses would show a much greater probability of arriving at the sensitive well; 2) the sensitive well would be more likely to have multiple occurrences of viruses; and 3) viruses would, in each case, arrive at the sensitive well at significantly higher concentrations.

An additional factor that impacts any sensitivity analysis is that virus sources are typically septic tanks. Bacterial sources can be septic tanks, farm animals or their manure, or wild animals. Thus, virus sources are less numerous and more likely to be located at a distance from a well. EPA recognizes that most States have sanitary setback distances that specify minimum protective distances between septic tank and well. However, most States do not vary protective setback distances based on ground water velocity or the aquifer sensitivity. Thus, EPA believes that septic tanks will be located at about the same distances from wells in both sensitive and non-sensitive aquifers.

The GWR also does not attempt to differentiate the relative hazard associated with shallow versus deep wells. EPA recognizes that shallow wells could have a greater probability of a virus occurrence and, if contaminated, a higher concentration. For example, Francy et al. (2004) identified enteric viruses in two shallow wells in non-sensitive Michigan aquifers. EPA believes that shallow wells in non-sensitive aquifers are a risk factor for fecal contamination.

## **6.4 Estimation of Occurrence of Indicators and Enteric Viral Pathogens**

This section discusses the estimation of the percentages of samples and wells that will be positive for viruses and indicators, along with the estimation of concentrations associated with positive samples.

Estimates are made for both viral<sup>12</sup> and indicator<sup>13</sup> “hit” (positive sample and positive well) rates, viral concentrations, and co-occurrence of viruses and indicators. Cost and benefit analyses performed for the economic analysis accompanying the GWR proposal drew data from two of the occurrence studies to inform the analyses— Lieberman et al. (2002) and Abbaszadegan et al. (2003). At the time, these two studies were considered to be the best suited for representing viral and indicator hit rates as well as viral concentration.

To improve the estimates of viral and indicator hit rates and concentrations using the data available, EPA convened a 2-day statistical workshop in May 2005. The core workgroup included expert participants from several government agencies and private consulting firms. The charge to the workgroup was to consider how to obtain improved modeling of:

- a) national viral occurrence in wells,
- b) indicator efficiencies for identifying fecally contaminated wells,
- c) indicator efficiencies for identifying virally contaminated wells, and
- d) virus concentrations in virus-positive well water.

By the end of the workshop, approaches for modeling viral and indicator prevalence and viral concentrations (items a, b, and d) were discussed, but methods for linking indicator occurrence and virus occurrence (item c) were not. Following the workshop, EPA acted on the workgroup’s recommendations, provided feedback to participants, and generated model-based national estimates for both viral and indicator occurrence. The results of this effort led naturally to a combined analysis, which also modeled co-occurrence of viruses and indicators. This combined model serves as the basis of EPA’s quantitative occurrence estimates. The sections below describe in detail how these new data are used to model the occurrence of virus and indicators in ground water sources.

The workgroup also considered the question of data selection with regard to the available occurrence studies. Individually, the studies are not nationally representative, but represent select portions of the ground water universe. Collectively, the studies describe a full range of geographic, geologic, and other characteristics (e.g., variety of system sizes and system types). Workshop participants recommended against discarding any study’s data without cause but did not feel they had the expertise to make any final calls regarding specific studies.

#### **6.4.1 Viral and Fecal Indicator Hit Rates**

This section discusses the calculation of hit rates for both viruses and indicators. The relationships between the occurrence of microbial water quality indicators and enteric pathogens have not been definitively established for environmental samples. However, indicators are still considered useful in assessing vulnerability of PWSs. Chapter 2 described the characteristics of ideal indicator organisms.

The rates are derived from pooled analyses of the data presented in Sections 6.2 and 6.3. Hit rate information is a critical input for both the model of baseline risk of viral infections, illnesses, and deaths

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<sup>12</sup> Although the GWR is aimed at preventing exposure to all viral pathogens, enterovirus data are used as a proxy for all viral pathogens in both the viral hit rate and viral concentration analyses.

<sup>13</sup> Although the GWR allows different indicators to be used for compliance purposes, *E. coli* is used as a proxy for all indicators in the hit rate analyses.

and for modeling the reduction of viral risks from components of the GWR dependent upon source water monitoring of fecal indicators.

The term hit rate refers to the probability that a virus or fecal indicator, or both, will ever be present in the source water of a well and, if so, how frequently each is expected to be present. Hit rates, therefore, have two components, which are referred to here as  $P_{\text{well}}$  and  $P_{\text{sample}}$ .

$P_{\text{well}}$  refers to the probability that a randomly selected well will ever have a virus (or indicator) present in its source water. Applying this probability to all wells provides an estimate of the number of wells that ever have viruses (or indicators) present in their source water.

$P_{\text{sample}}$  refers to the probability that a random sample from a contaminated well will be positive. This value varies from well to well, as some wells have contamination present more frequently than others.

So, for example, a virus  $P_{\text{well}}$  value of 0.10 implies that 1 out of every 10 wells will have detectable virus present in its source water at some time. Conversely, it also implies that 9 out of 10 wells will not ever have detectable viruses.

A well with a virus  $P_{\text{sample}}$  value of 0.25 would be expected to have detectable viruses in 1 of every 4 samples assayed.

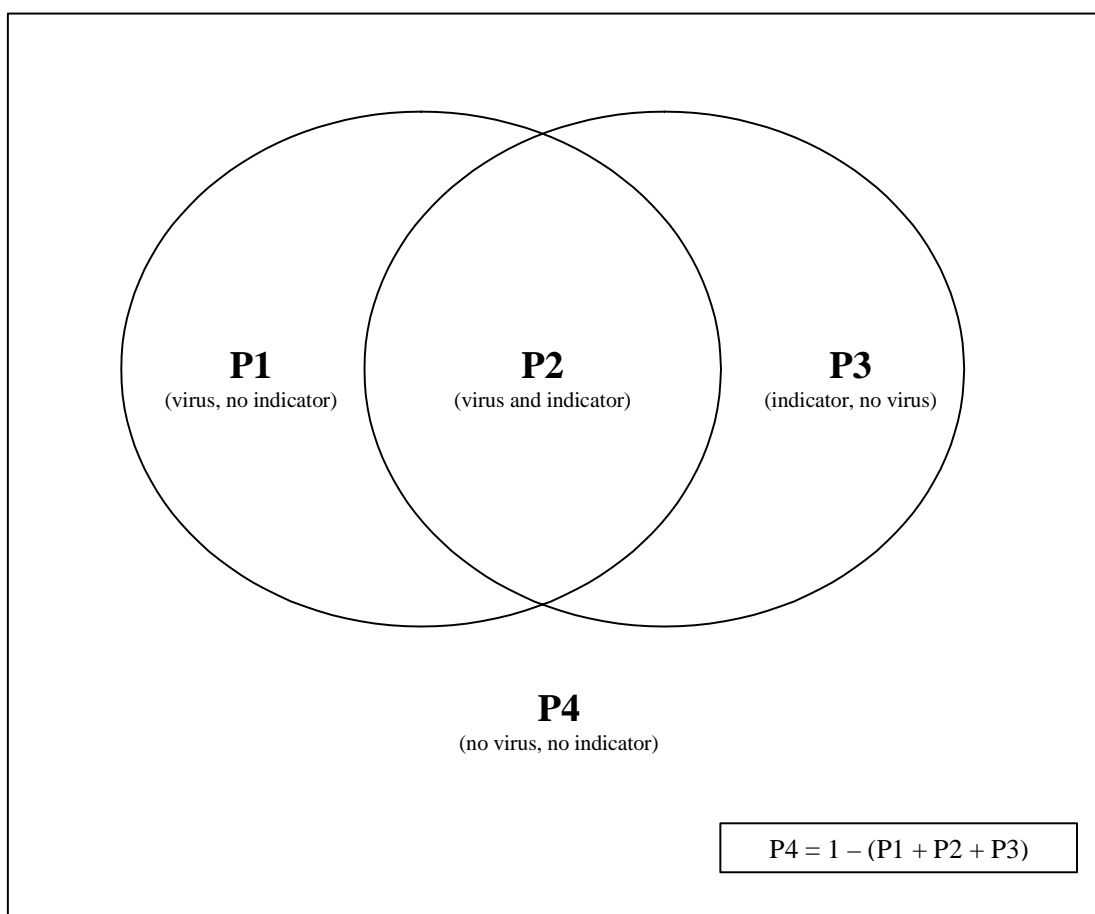
There are a number of factors that influence the estimation, as well as the interpretation, of  $P_{\text{sample}}$ . Microorganisms in water are dispersed spatially at low average concentrations relative to the volumes of water typically collected in assays. As a result, a randomly taken sample of some volume  $V$  may not have the microorganism present even when it is known to be in the source water. Often the recovery rate for these pathogens is less than 100 percent, and viruses that are present in samples are not always detected. In addition, the actual presence of microorganisms in the source water is recognized as being intermittent in nature due to changes in the actual sources of the contamination as well as hydrogeological and other physical factors affecting transport from the sources to the water used at that well.

It is important to recognize that while the  $P_{\text{well}}$  value applies to all wells, each individual contaminated well is expected to have its own  $P_{\text{sample}}$  value. That is, the underlying data suggest that among those wells that have a virus or fecal indicator present at some time, the probability of observing it in a given sample (that is, of it being present in that sample volume on that particular day) will vary from well to well. Consequently, a distribution of  $P_{\text{sample}}$  values was derived to reflect  $P_{\text{sample}}$  variability from well to well. Specifically, a beta distribution of  $P_{\text{sample}}$  was derived from the underlying occurrence data. The beta distribution is often used for this purpose, that is, to describe distributions of probabilities or other variables that range between zero and one. (The probit and logit distributions are sometimes used for this purpose, and generally produce estimates similar to those produced using the beta distribution.) The beta distribution is a two-parameter distribution; the parameters are usually designated  $\alpha$  and  $\beta$ . The estimation of those parameters for the beta distribution of  $P_{\text{sample}}$  is described further below.

The Venn diagram shown in Exhibit 6.4 describes the basic co-occurrence model. This diagram shows that some fraction of wells ( $P_1$ ) has some virus contamination, but no indicator, while another fraction of wells ( $P_2$ ) has both virus and indicator, and a third fraction of wells ( $P_3$ ) has indicator, but no viral occurrence. A fourth fraction of wells ( $P_4$ ), having neither viral nor indicator occurrence, is the remainder:  $P_4 = 1 - (P_1 + P_2 + P_3)$ .



## Exhibit 6.4 Venn Diagram for Universe of Ground Water Wells



To fully characterize both  $P_{\text{well}}$  and  $P_{\text{sample}}$  for viral pathogens and fecal indicators, and to characterize their co-occurrence, the model requires the estimation of seven parameters:  $P1$ ,  $P2$ ,  $P3$ ,  $\alpha_v$  (for viruses),  $\beta_v$ ,  $\alpha_i$  (for indicators), and  $\beta_i$  from the available occurrence data.  $P_{\text{well}}$  for virus is equal to  $P1+P2$ , and  $P_{\text{well}}$  for indicators is equal to  $P2+P3$ .  $P_{\text{sample}}$  for viruses (referred to hereafter as  $P_{\text{sample } v}$ ) at different wells is described by a beta distribution with the parameters  $\alpha_v$  and  $\beta_v$ . Similarly,  $P_{\text{sample}}$  for indicators (referred to hereafter as  $P_{\text{sample } i}$ ) at different wells is described by a beta distribution with the parameters  $\alpha_i$  and  $\beta_i$ .

It is important to note that the occurrence model developed by EPA relates virus and indicator co-occurrence only in terms of  $P_{\text{well}}$  (the fraction of wells having one, the other, or both). It does not provide for different levels of  $P_{\text{sample } v}$  or  $P_{\text{sample } i}$  in wells having both or only one of the two contaminants. Wells having both virus and indicator presence may well have them more often than wells having only viruses or wells having only indicators. A model that includes this feature would require additional parameters to correlate  $P_{\text{sample } v}$  and  $P_{\text{sample } i}$  in wells having both virus and indicator. However, the limited amount of occurrence data is not sufficient for a model with that degree of complexity.

Another important point to note is that, while the preceding overview of the occurrence model refers to the  $P1$ ,  $P2$ , and  $P3$  parameters for  $P_{\text{well}}$  and the  $\alpha$  and  $\beta$  parameters for  $P_{\text{sample}}$  as though only single “best values” are estimated, the occurrence model is actually designed to capture the uncertainty in

those values and produces a very large number (10,000) of sets of those seven parameters. These sets of seven parameters are subsequently sampled in the Monte Carlo simulations that are performed for both the risk/benefits model and the cost model used to evaluate the impact of the ground water rule options.

### **Parameter Estimation Methods**

Markov Chain Monte Carlo (MCMC) methods were used in a Bayesian framework to produce samples from the joint posterior parameter distribution (the sample of 10,000 discussed in the paragraph above). This posterior density function is a product of a prior density function and a likelihood function. WinBUGS software (Gilks and Spiegelhalter 1994) was used to produce the large MCMC sample, which, in turn was used to inform the GWR risk and cost analyses. This section describes the prior and likelihood functions of the seven-parameter model.

#### *Non-Informative Priors*

Parameters P1, P2, and P3 [together with P4, where  $P4 = 1 - (P1 + P2 + P3)$ ] are the fractions of all ground water wells falling into the four possible subsets as shown in Exhibit 6.4. A relatively non-informative prior on these is Dirichlet with parameters (1, 1, 1, 1). This is the multivariate extension of the beta (1, 1) distribution, which is often used for the one-parameter case. Beta (1, 1) is a uniform distribution for one unknown over the range [0, 1] and likewise, Dirichlet (1, 1, 1, 1) is uniform over the three-dimensional space where the sum of P1, P2, and P3 is in the range [0, 1].

$P_{\text{sample } v}$  and  $P_{\text{sample } i}$  are both assumed to be beta-distributed across wells having virus presence and wells having indicator presence, respectively. The Beta density function is usually expressed in terms of its parameters  $\alpha$  and  $\beta$  as:

$$\text{dbeta}(p, \alpha, \beta) = p^{\alpha-1} \cdot (1-p)^{\beta-1} \cdot \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha) \cdot \Gamma(\beta)}$$

where  $p$  in this expression is one of the  $P_{\text{sample}}$  variables. Assigning priors with this form of the density is difficult. First, it is difficult because one cannot think about  $\alpha$  (or  $\beta$ ) without understanding  $\beta$  (or  $\alpha$ ). Second, a non-informative prior leads to an improper posterior density, with increasing mass as the sum  $(\alpha + \beta)$  becomes large. In our case, a uniform prior on  $(\alpha, \beta)$  suggests strong knowledge that the variability of  $P_{\text{sample}}$  is small about some mean value. Gelman et al. (1995) discuss this problem in their book *Bayesian Data Analysis* and suggest reparameterization in terms of the mean,  $a = \alpha / (\alpha + \beta)$  and the inverse square root of the “sample size,”  $b = 1/(\alpha + \beta)^{0.5}$ . EPA adopted this parameterization and utilized disperse uniform priors for the two new parameters ( $a$  and  $b$ ). The conventional beta distribution parameters can be derived from new parameters  $a$  and  $b$  as follows:

$$\begin{aligned}\alpha &= a / b^2 \\ \beta &= (1 - a) / b^2\end{aligned}$$

Therefore, for parameterization of the occurrence model,  $a_v$ ,  $b_v$ ,  $a_i$ , and  $b_i$  are estimated, and the corresponding  $\alpha$  and  $\beta$  values for the beta distributions are computed from them as shown above.

## The Likelihood Function

The virus and indicator data for an individual well used as input to EPA's occurrence model can be reduced to four integers. The four integers for a well are:

$N_v$  = the total number of virus assays for the well  
 $K_v$  = the number of virus positives for the well  
 $N_i$  = the total number of indicator assays for the well  
 $K_i$  = the total number of indicator positives for the well

Up to three of these may be zeros; at least one of the values  $N_v$  or  $N_i$  must be  $\geq 1$  for it to be valid input to the model.

The likelihood of a well's data, given parameter values ( $P1$ ,  $P2$ ,  $P3$ ,  $\alpha_v$ ,  $\beta_v$ ,  $\alpha_i$ , and  $\beta_i$ , and the category of a well), is a function of the parameter values and the well's data (the well's  $N_v$ ,  $K_v$ ,  $N_i$ , and  $K_i$  values), where  $\alpha_v$  and  $\beta_v$  are parameters for beta-distributed  $P_{\text{sample } v}$  for viruses and  $\alpha_i$  and  $\beta_i$  are parameters for beta-distributed  $P_{\text{sample } i}$  for indicators. The total likelihood (for the entire data set) is simply the product of these individual well likelihoods.

In general, the likelihood for a well has three parts, the probability of what was observed for virus, given the number of virus assays, the probability of what was observed for *E. coli*, given the number of *E. coli* assays, and the probability of the well's membership in its category ( $P1$ ,  $P2$ ,  $P3$ , or  $P4$ ). Below, these two factors are defined for wells of the four different categories (virus only, virus and *E. coli*, *E. coli* only, and no contamination):

1. Well has some virus occurrence, but no *E. coli* occurrence (in area P1 of Exhibit 6.4):

$$L1(K_v, N_v) = P1 \cdot \int_0^1 \text{dbeta}(P_{\text{sample } v}, \alpha_v, \beta_v) \cdot \text{dbinom}(K_v, N_v, P_{\text{sample } v}) dP_{\text{sample } v}$$

where dbeta is the beta probability density function and dbinom is the binomial probability mass function. A well of this type must have had no *E. coli* detections, so the probability of observing  $K_i = 0$  positives is 1 and its product with  $L1(K_v, N_v)$  is simply  $L1(K_v, N_v)$ .

2. Well has both virus and *E. coli* occurrence (in area P2 of Exhibit 6.4):

$$L2(K_v, N_v, K_i, N_i) = P2 \cdot L1(K_v, N_v) \cdot \int_0^1 \text{dbeta}(P_{\text{sample } i}, \alpha_i, \beta_i) \cdot \text{dbinom}(K_i, N_i, P_{\text{sample } i}) dP_{\text{sample } i}$$

Note that, to be in this category, it is not necessary that a well actually have observed virus and *E. coli* positives. Having no positive, based on a small number of assays, is only weak evidence that a well belongs to another category. At each uncertainty iteration, wells are assigned to categories according to the likelihood, conditional on the well's data plus all other parameter values at that time. In this fashion, parameters  $P1$ ,  $P2$ ,  $P3$ , and  $P4$  also enter the likelihood. The only wells that are assigned to this category with certainty are those which were observed to be positive for both viruses and *E. coli*. At every

iteration, they are placed in this category. All other wells are randomly assigned to different categories from iteration-to-iteration, according to their likelihoods.

3. Well has *E. coli*, but no virus occurrence:

$$L_3(K_i, N_i) = P_3 \cdot \int_0^1 \text{dbeta}(P_{\text{sample}_i}, \alpha_i, \beta_i) \cdot \text{dbinom}(K_i, N_i, P_{\text{sample}_i}) dP_{\text{sample}_i}$$

4. Well has neither virus nor *E. coli* occurrence:

Wells having no observed contamination can belong to any category. Wells assigned to this category must always have negative assays. The likelihood of observing no positives is certain, so the only contribution to the likelihood is the probability of membership, P4.

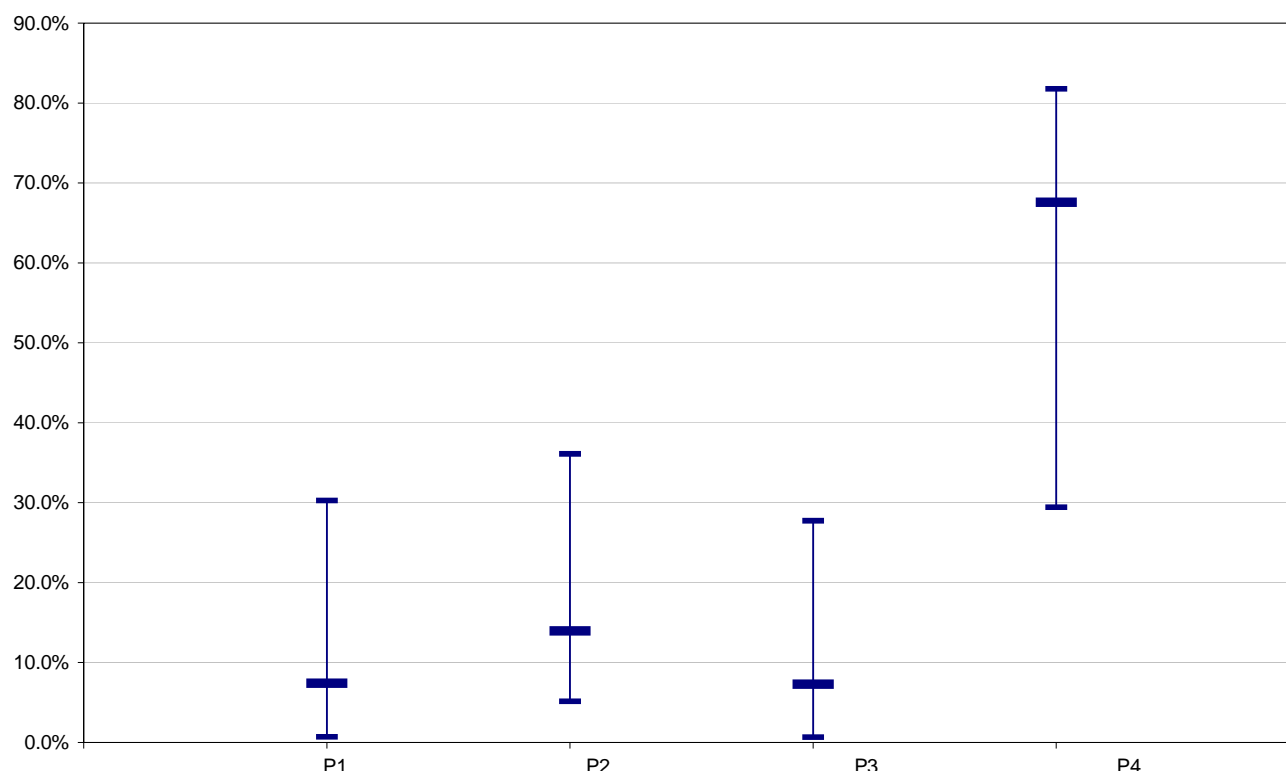
#### *Estimates for Combined Model*

Estimates were produced by Markov Chain Monte Carlo methods using WinBUGS software (Gilks and Spiegelhalter 1994). An important feature of this MCMC analysis is that it produces a large, well-mixed sample of outputs wherein each individual output contains a plausible value for each of the seven parameters in combination with one another. In this modeling, EPA captured 10,000 sets of results for the seven parameters to characterize uncertainty about the parameter values. The MCMC modeling captures the uncertainty in the parameter estimates through this large number of sets of results with appropriate correlation structure.

The actual data used to estimate the seven parameters are the enteroviruses cell culture data (for viruses) and the *E. coli* data (for indicators) from the 15 occurrence studies described in Section 6.2. Of the 15 studies, 12 have enterovirus cell culture data and 12 have *E. coli* data. Data from GWUDI wells were excluded from the analysis.

The following exhibits provide summaries of the  $P_{\text{well}}$  and  $P_{\text{sample}}$  results obtained from the modeling for viruses and indicators. Exhibit 6.5 shows the median values for P1, P2, P3, and P4. The “error bars” included on the graphs reflect the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the 10,000 values estimated. As indicated earlier (refer to Exhibit 6.4), P1 refers to the fraction of wells having viruses at some time (but no indicator), P2 refers to those wells having viruses and an indicator at some time, and P3 refers to those having an indicator at some time (but no virus). P4 are those wells having neither virus nor indicator occurrence.

**Exhibit 6.5 Median of 10,000 Estimates of P1, P2, P3, and P4  
(with Error Bars Showing the 5th and 95th Percentiles)**

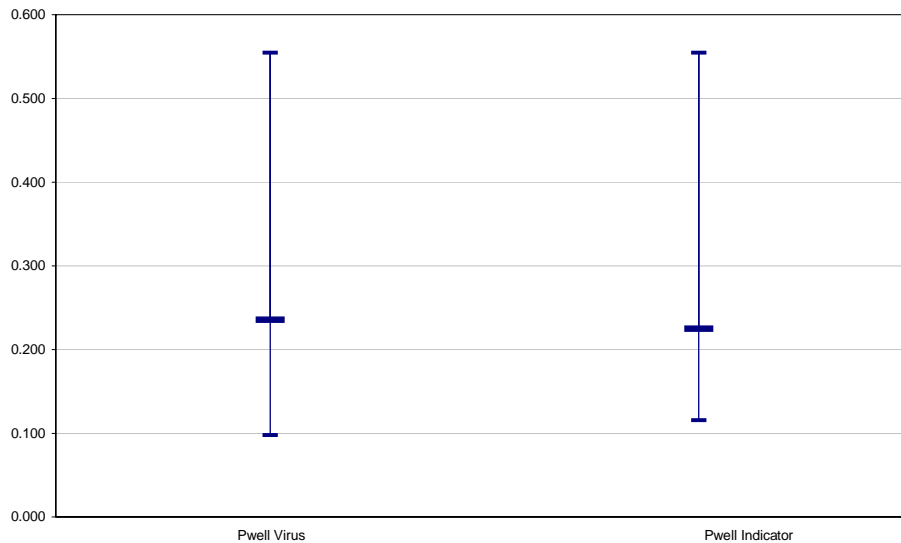


The median values obtained in the model for P1, P2, and P3 are 7.4 percent, 14.0 percent and 7.3 percent, respectively. The median value for the sum P1+P2+P3 (wells with sometime presence of virus and/or indicator) is 32.4 percent. The 5<sup>th</sup> and 95<sup>th</sup> percentiles on the sum of P1, P2, and P3 were found to be 18.2 percent and 70.6 percent. P4, the remaining wells that have neither virus nor indicator present at anytime is derived from the model estimates for the other three as 1 minus (P1+P2+ P3). The median P4 value is 67.6 percent, with 5<sup>th</sup> and 95<sup>th</sup> percentiles on P4 of 29.4 percent and 81.8 percent. Thus, approximately 90 percent of the 10,000 estimates of wells with either virus or fecal indicator occurrence fall between about 20 percent and 70 percent, with a central estimate of about 32 percent.

If *E. coli* was a perfect indicator of virus occurrence, there would be no wells with only virus or only *E. coli*. P1 and P3 would both be zero. Clearly, *E. coli* is not a perfect indicator of viral occurrence. Exhibit 6.5 shows that most wells with virus occurrence tend to also have *E. coli* occurrence (P2 is greater than P1) and that most wells with *E. coli* occurrence tend to also have virus occurrence (P2 is greater than P3). Given that approximately 24 percent of wells have virus occurrence while 23 percent of wells have *E. coli* occurrence, if viruses and *E. coli* were completely independent, then the fraction of wells having both (P2) would equal the product 0.24 \* 0.23, or 5.5 percent. The large median value of P2 (14.0 percent) demonstrates that, though imperfect, *E. coli* is a positive indicator of viral occurrence.

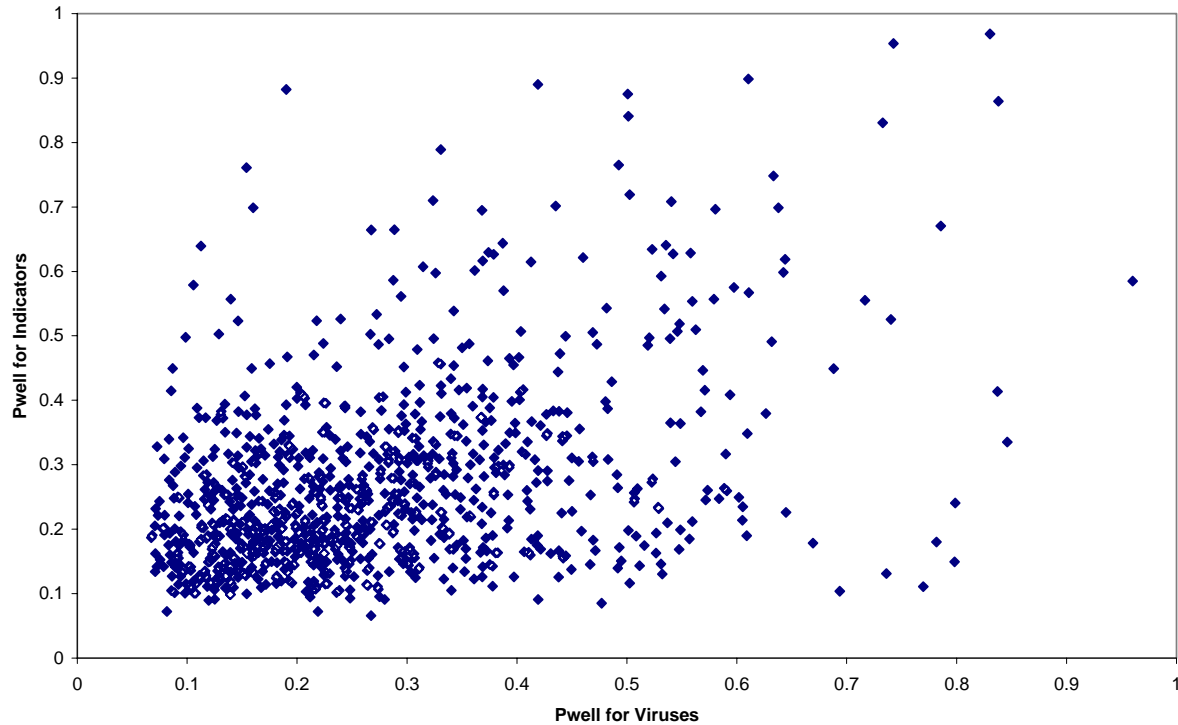
As noted previously, two of the important hit rate values are  $P_{well}$  for viruses and  $P_{well}$  for indicators. These are composed of P1+P2 for viruses and P2+P3 for indicators. Exhibit 6.6 provides the median (and the 5<sup>th</sup> and 95<sup>th</sup> percentile values) for  $P_{well}$  for viruses and for indicators.

**Exhibit 6.6 Median of 10,000 Estimates of  $P_{\text{well}}$  for Virus and Indicator  
(with Error Bars Showing the 5th and 95th Percentiles)**



The median value of  $P_{\text{well}}$  for viruses was found to be 23.6 percent with 5<sup>th</sup> and 95<sup>th</sup> percentiles of 9.8 percent and 55.5 percent. The median of  $P_{\text{well}}$  for indicators was 22.5 percent with 5<sup>th</sup> and 95<sup>th</sup> percentiles of 11.6 percent and 55.5 percent. As shown in Exhibit 6.6, median values and overall ranges of  $P_{\text{well}}$  for viruses and indicators are quite similar. However, the distribution of paired values for these covers a very wide range of combinations. The scatter plot shown in Exhibit 6.7 shows the paired combinations of a sample of 1,000 of the 10,000 values. While most of the pairs tend to fall in the 10 to 20 percent range for both viruses and indicators, there are a substantial number that fall above this range, including many where one value for the pair is high and the other relatively low.

**Exhibit 6.7 Scatter Plot of  $P_{\text{well}}$  Pairs for Indicators and Viruses**

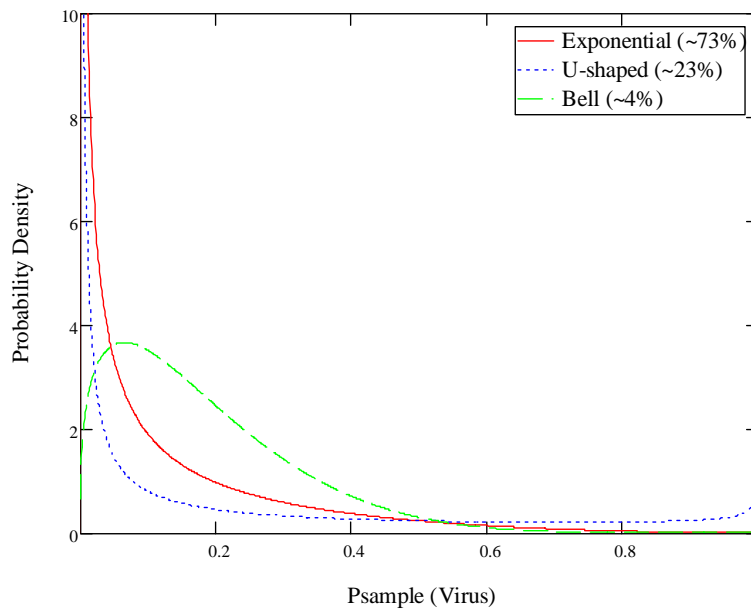


As described above, the  $P_{\text{sample } v}$  for viruses and  $P_{\text{sample } i}$  for indicators are not single value estimates but are, rather, distributions of values reflecting the variability in  $P_{\text{sample}}$  from well to well. As a result, the occurrence model generates 10,000 of these distributions for both  $P_{\text{sample } v}$  and  $P_{\text{sample } i}$ . It is difficult to provide a summary of all 10,000 of those distributions, particularly because the beta distribution used in this analysis can take on a wide range of shapes.

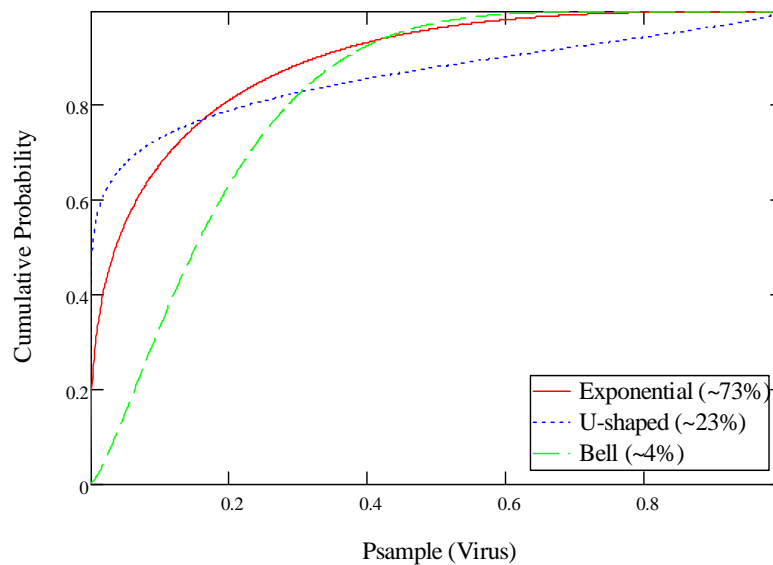
The beta distributions obtained for  $P_{\text{sample}}$  have three different shapes: exponential, U-shaped, and bell-shaped (right-skewed). Representative examples of these three shapes for  $P_{\text{sample}}$  for viruses are presented in Exhibit 6.8 as the density functions and in Exhibit 6.9 as the cumulative probability distributions. (Note that these particular examples were selected because they present values that are close to the central tendencies for the three distribution shapes of  $P_{\text{sample}}$  for viruses.)

For  $P_{\text{sample}}$  for viruses, about 73 percent of the distributions have the exponential shape, 23 percent have the U-shape, and 4 percent have the right-skewed bell shape. For  $P_{\text{sample}}$  for indicators, about 79 percent of the distributions have the exponential shape, 2 percent have the U-shape, and 19 percent have the right-skewed bell shape.

**Exhibit 6.8 Density Function Shapes of  $P_{\text{sample}}$  for Viruses**



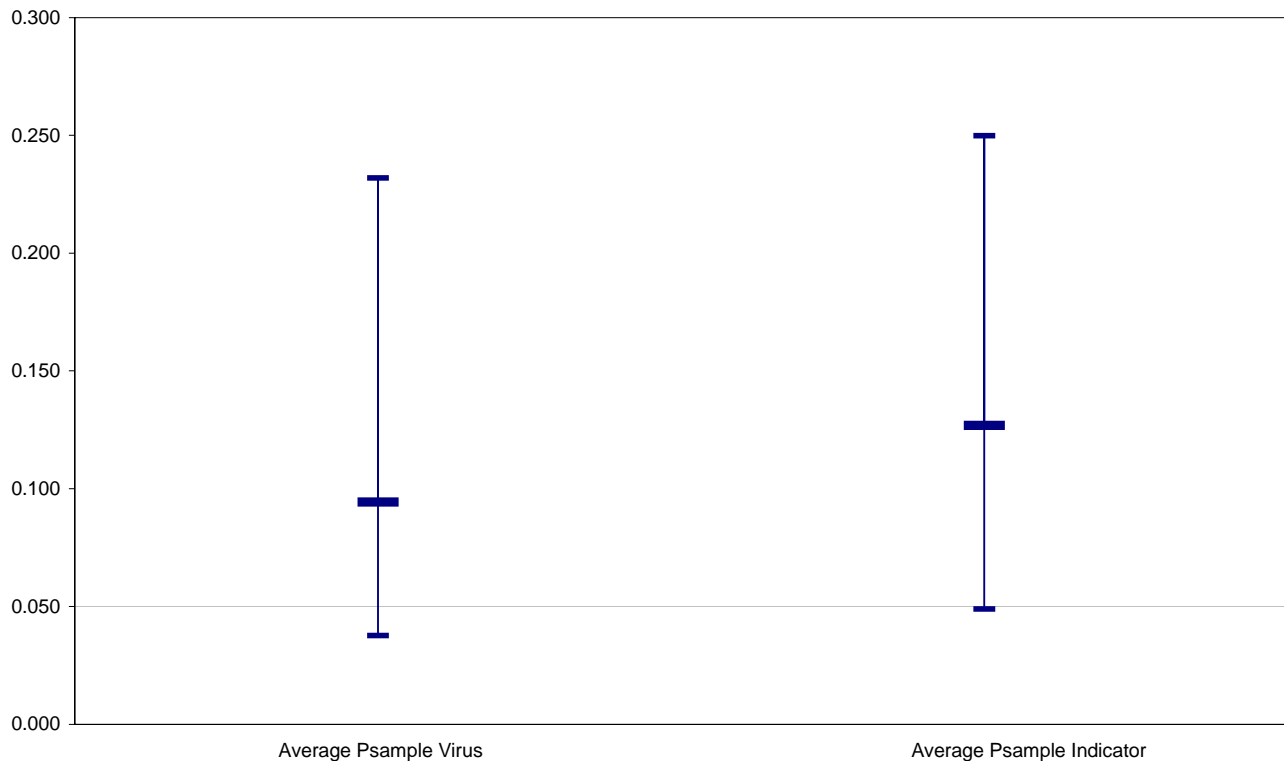
**Exhibit 6.9 Cumulative Distributions of  $P_{\text{sample}}$  for Viruses**



One way to summarize the full set of 10,000  $P_{\text{sample}}$  distributions generated by the occurrence model is in terms of the range and central tendency of their expected values. For  $P_{\text{sample } v}$ , the median of the expected values is 9.4 percent, with 5<sup>th</sup> and 95<sup>th</sup> percentile values of 3.8 percent and 23.2 percent, respectively. For  $P_{\text{sample } i}$ , the median of the expected values is 12.7 percent, with 5<sup>th</sup> and 95<sup>th</sup> percentile values of 4.9 percent and 25.0 percent, respectively. These values are also shown graphically in Exhibit 6.10.

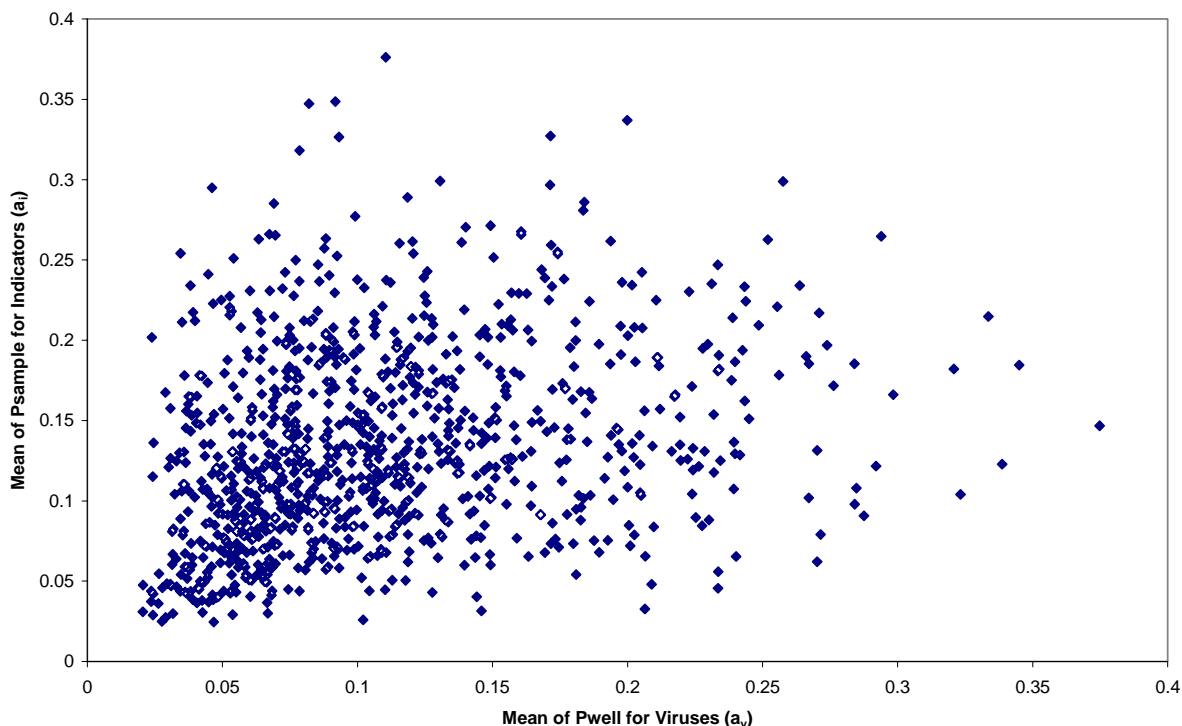


**Exhibit 6.10 Median of 10,000 Estimates of  $P_{\text{sample}}$  for Virus and Indicator  
(with Error Bars Showing the 5th and 95th Percentiles)**



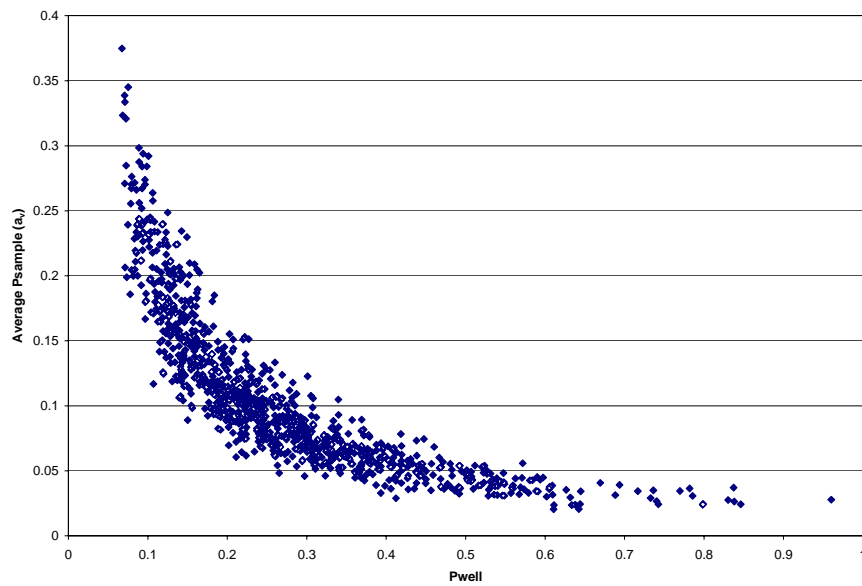
The range and central tendency of the expected values for  $P_{\text{sample } v}$  and  $P_{\text{sample } i}$  are similar. The distribution of expected values for  $P_{\text{sample } v}$  and  $P_{\text{sample } i}$  pairs produced by the model, shown in Exhibit 6.11 for a sample of 1,000 pairs, shows a substantial number of pairs where both values are in the 5 to 15 percent range. However, there are a number where one of the pair is substantially higher (or lower) than the other member of the pair.

**Exhibit 6.11 Scatter Plot of Means of  $P_{\text{sample}}$  Pairs for Indicators and Viruses**

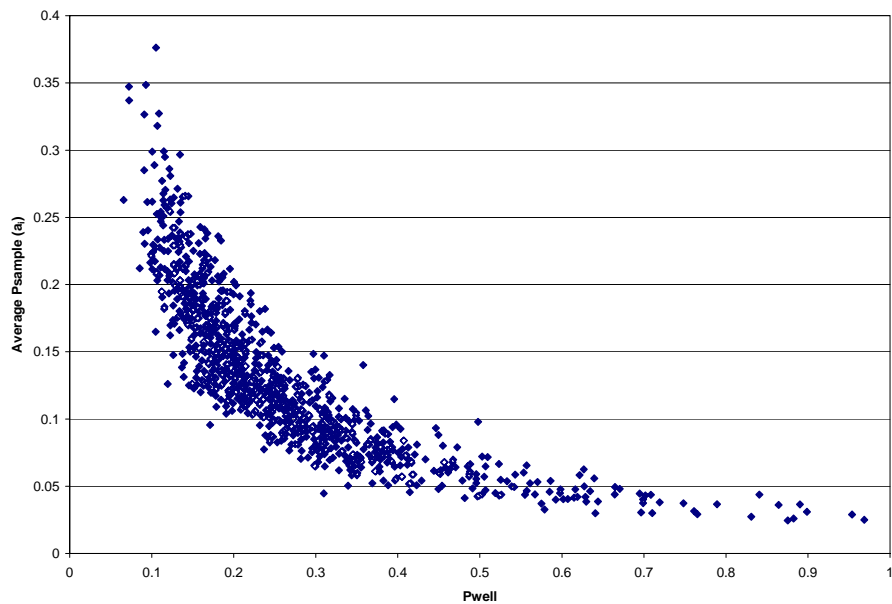


An important relationship that can be seen from the results of the occurrence modeling is the one between paired  $P_{\text{sample}}$  and  $P_{\text{well}}$  values. For both viruses and indicators, it was found that there is, generally, an inverse relationship between them. The product of  $P_{\text{well}}$  and the average of  $P_{\text{sample}}$  is approximately equal to the overall fraction of samples found to be virus positive, therefore the inverse relationship is expected. If either  $P_{\text{well}}$  or the average  $P_{\text{sample}}$  were increased without increasing the other, then significantly more virus-positive results should have been observed across the survey data sets. Similarly, a decrease in one, but not the other, would predict fewer positives than were observed. That is, a characteristic of the uncertainty revealed by the 10,000 sets of results from the occurrence modeling is that if the ‘true’ value of  $P_{\text{well}}$  (the fraction of wells that have virus or indicator present at some time) is high, the chance of finding the organism in a given sample at those wells tends to be low. Conversely, if the ‘true’ value of  $P_{\text{well}}$  is low, the chance of finding the organism in a given sample at those wells tends to be higher. These relationships are shown in Exhibits 6.12 and 6.13 for viruses and indicators, respectively, for a sample of 1,000 from the 10,000 sets of results produced by the occurrence model.

**Exhibit 6.12 Mean of  $P_{\text{sample}}$  Versus  $P_{\text{well}}$  for Viruses  
(1,000 Pairs from Occurrence Model)**



**Exhibit 6.13 Mean of  $P_{\text{sample}}$  Versus  $P_{\text{well}}$  for Indicators  
(1,000 Pairs from Occurrence Model)**



## 6.5 Estimates of Well Vulnerability

### 6.5.1 Background

In the economic analysis for the proposed rule EPA estimated that 17 percent of the wells in the United States were improperly constructed and that 83 percent of the wells were properly constructed (ASDWA 1997). EPA used the Lieberman et al. (2002) data set to represent viral occurrence in improperly constructed wells and the Abbaszadegan et al. (2003) data set to represent properly constructed wells. It was implied that well construction corresponded with vulnerability (i.e., poorly constructed wells would be vulnerable to contamination). EPA received public comments on the proposal that questioned the basis for using Lieberman et al. data to represent improperly constructed wells because the Lieberman study sites were chosen based on the presence of total coliforms and indicators of fecal contamination, rather than on well construction documents. To clarify this issue, EPA is categorizing ground water systems into two groups: those that are more vulnerable and those that are less vulnerable.<sup>14</sup> Neither of the studies discussed above provide data regarding the percentage of ground water sources that might be more or less vulnerable, and EPA needed to derive such estimates to support the economic analysis for the GWR. EPA decided to use national data on Total Coliform Rule (TCR) violations to estimate the percent range of wells that are more or less vulnerable. The proportion of wells in each of the well vulnerability categories is necessary to properly apportion the virus concentration data. Viral concentration data from wells with a history of total coliform contamination (i.e., the Lieberman et al. 2002 data) are used for the wells that are identified as belonging within the more vulnerable group. Following is a description of these estimates and their bases.

### 6.5.2 Estimating Percentage of Wells in Vulnerability Categories

EPA categorized systems into two groups: those that are more vulnerable and less vulnerable.

*More vulnerable systems:* These are systems that may be more vulnerable to source water contamination, as reflected by maximum contaminant level (MCL) violations under the TCR during a calendar year (from SDWIS, USEPA 2003a).

*Less vulnerable systems:* These are systems that are expected to be less vulnerable to source water contamination, reflected by having *not* had an MCL violation under the TCR during the same year.

The percentage of systems in the “more vulnerable” category (and also the percentage in the “less vulnerable” category) varies by system type (i.e., community, non-transient community, and transient non-community) and system size, and ranges from zero to 6.83 percent. The proportions of wells in the more vulnerable category are identified in Exhibit 6.14. For each element in the exhibit (system size and type) the proportion of less vulnerable wells is 100 percent minus the value identified in the exhibit. Detail on the derivation of these percentages is presented in Exhibit 2.5 in Chapter 2 and in Appendix B of the *Economic Analysis for the Final Ground Water Rule*.

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<sup>14</sup> EPA believes this terminology is more appropriate than that used in the proposal (“improperly constructed” and “properly constructed”) since the Lieberman et al (2002) study did not target poorly constructed wells, but rather used criteria believed to favor the selection of vulnerable wells.

**Exhibit 6.14 Mean Percent of Systems with Acute or Monthly MCL Violations  
by System Type and System Size**

System Size (Population Served)	CWS	NTNCWS	TNCWS
<100	2.87%	2.84%	2.33%
101-500	2.66%	2.25%	2.40%
501-1,000	1.85%	1.86%	2.30%
1,001-3,300	2.23%	2.34%	3.59%
3,301-10,000	3.48%	2.21%	2.82%
10,001-50,000	3.41%	0.00%	2.94%
50,001-100K	1.82%	0.00%	0.00%
100,001- 1 Million	3.13%	0.00%	0.00%
> 1 Million	0.00%	0.00%	0.00%

Source: Appendix B of the Economic Analysis for the Final GWR

For ground water systems, the violation of MCLs under the TCR is an indicator of vulnerability, especially when systems do not disinfect and distribution systems are small or do not exist. There is some uncertainty associated with the data in Exhibit 6.14 because they include systems that disinfect as well as those that do not disinfect. Exhibit 6.15 summarizes the available data on disinfecting systems; 64 percent of ground water systems provide no disinfection. For such systems, positive samples under the TCR may reflect contamination in their source water. In transient non-community systems, which essentially have no distribution systems, 82 percent of systems provide no disinfection. In these systems, the influence of non-source water-related contamination is likely to be very low relative to that of source water. In summary, it is assumed that disinfection has only a small influence on the identification of more vulnerable wells using TCR violation data.

**Exhibit 6.15 Number and Percent of Systems Disinfecting,  
By Type of System**

	Total	CWS	NTNCWS	TNCWS
Number of Systems	147,330	42,361	18,908	86,061
Approximate Percentage of Systems Disinfecting	36%	75%	29%	18%

Source: Derived from Exhibit 4.2 of the Economic Analysis for the Final Ground Water Rule

## **6.6 Estimates of Occurrence and Concentrations**

### **6.6.1 Use of Indicator Occurrence for Assessment and Triggered Monitoring**

This section describes how the occurrence modeling described above, specifically that for the indicator hit rates, is used to predict the number of wells “captured” by triggered source water monitoring and source water assessment monitoring. The fraction of wells producing an indicator positive upon their first assay (whether as part of an assessment or triggered monitoring program) can be estimated as a

function of the following:

$P_{i,\text{well}}$  = fraction of wells with some indicator occurrence  
 $\alpha_i$  = first parameter of beta-distributed  $P_{\text{sample } i}$   
 $\beta_i$  = second parameter of beta-distributed  $P_{\text{sample } i}$

As discussed in the section above, in each uncertainty iteration of the occurrence model, a set of parameter values describing indicator occurrence is selected from the MCMC sample (as well as parameters for  $P_{v,\text{well}}$  and  $P_{\text{sample } v}$  that describe virus occurrence). The probability that an indicator positive will be observed by the time of the  $i^{\text{th}}$  assay can be obtained from  $F_{n,i}$ :

The probability that the  $i^{\text{th}}$  assay will be the very first positive for the site is the difference  $F_{n,i} - F_{n,i-1}$ . This is, then, the fraction of all wells expected to return an indicator positive upon the  $i^{\text{th}}$  assay.

These probabilities (the  $F_n$  values) were derived for assays  $i = 1$  through 200 for  $n = 10,000$  uncertainty iterations. These probabilities are specifically associated with each set of the seven occurrence parameters generated by the model as described previously.

Exhibit 6.16 shows the cumulative probability of having an indicator on or before the indicator assay number. A sample of 1,000 sets was generated from the occurrence model, and three of the 1,000 curves are shown in the graph corresponding to the 5<sup>th</sup> percentile, median, and 95<sup>th</sup> percentile of all values for that assay number. These data are used in the cost model simulation, discussed further in the economic analysis, to determine whether, and if so, when a given well conducting source water monitoring (either triggered or assessment monitoring) will have its first indicator positive and as a result initiate corrective action.

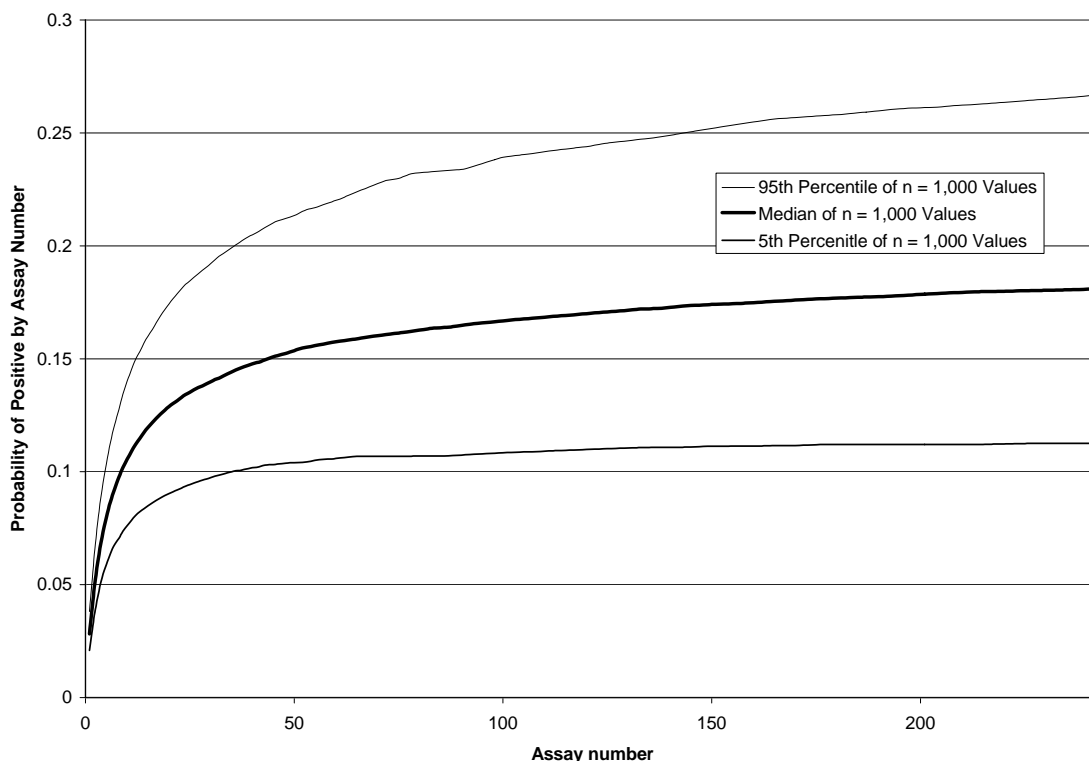
$$F_{n,i} = P_{i,\text{well}} \cdot \int_0^1 \text{dbeta}(P_{\text{sample } i}, \alpha_n, \beta_n) \cdot \left[ 1 - (1 - P_s)^{i+1} \right] d P_s$$

which simplifies to:

$$F_{n,i} = P_{i,\text{well}} - P_{i,\text{well}} \cdot \frac{\Gamma(\alpha_n + \beta_n) \cdot \Gamma(\beta_n + i)}{\Gamma(\alpha_n + \beta_n + i) \cdot \Gamma(\beta_n)}$$

These data suggest that of all wells taking source water indicator samples, just under 20 percent would be expected to have a positive result on or before the 200th assay, as a central tendency estimate, with an uncertainty range from approximately 10 percent to over 25 percent.

## Exhibit 6.16 Cumulative Probability of an Indicator Positive as a Function of Assay Number -- All Wells (Used for Cost Analysis)

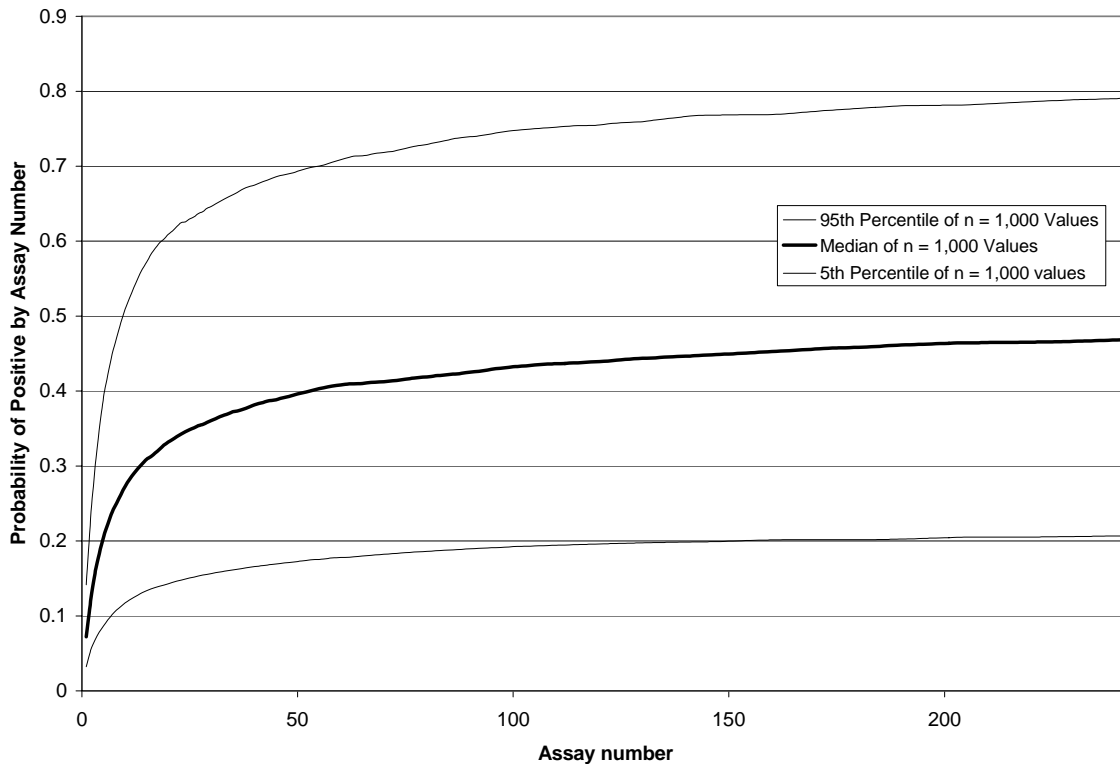


Similar data on the occurrence of the first indicator positive as a function of assay number are used in the risk reduction model, to determine the effectiveness of indicator monitoring in source water to “capture” wells that are known to have virus present at some time (that is, areas P1 + P2 in the Venn diagram shown earlier in this chapter). For this part of the analysis, the  $F_{n,i}$  values are adjusted to account for assays performed on those wells that are in the P1 + P2 “space.” The adjustment made to the value of each assay probability result obtained as shown above is to multiply it by:

$$\frac{P2}{(P1 + P2) \cdot (P2 + P3)}$$

Exhibit 6.17 shows the three corresponding distributions for these adjusted values used for the risk reduction modeling. These data suggest that of those wells that sometimes have viruses present and that have data for source water indicator samples, just under 50 percent would be expected to have a positive result on or before the 200<sup>th</sup> assay, as a central tendency estimate, with an uncertainty range from approximately 20 percent to 80 percent. The higher values shown here relative to the “all wells” data shown above reflect the outcome that a much higher proportion of wells having some time virus presence also have some time indicator presence [i.e.,  $P2/(P1+P2)$  than do all wells [i.e.,  $(P2+P3)/(P1+P2+P3+P4)$ ].

### Exhibit 6.17 Cumulative Probability of an Indicator Positive as a Function of Assay Number -- Virus Positive Wells (Used for Risk Reduction Analysis)



It is important to note also that both of these sets of results indicate that observing an indicator positive in an early assay is more likely than on a later assay. This is because the structure of the model accounts for the higher likelihood of observing positives among those wells where the frequency of occurrence (that is,  $P_{\text{sample}}$ ) is greatest.

#### 6.6.2 Pathogen Concentration Analysis

The preceding section addressed hit rates, which comprise the first aspect of characterizing virus occurrence in source water used by public ground water wells. This section addresses virus concentrations, which comprise the second aspect of occurrence.

Hit rates primarily address the presence or absence of virus in the water. The two components of hit rates are  $P_{\text{well}}$ , which characterizes the fraction of wells where viruses are either present at some time or are never present, and  $P_{\text{sample}}$ , which characterizes the fraction of samples or duration of time that the organisms occur in those wells that have viruses at some time.

If a well is one in which the virus is never detected (which is expected to be the case the majority of the time), the virus concentration is assumed to be zero. For those wells at which viruses are present at detectable levels, it is necessary to characterize the expected concentrations of viruses so that the baseline



risk and the risk reductions from regulatory alternatives can be estimated.<sup>15</sup>

The available information on virus concentrations in wells is limited. Useful information on virus concentrations are only available from cell culture results for enteroviruses. For the purposes of this analysis, it has been assumed that the concentrations of enteroviruses are similar to other viruses of concern.

Just as there is variability in virus occurrence with respect to prevalence, so too, there is variability in expected concentrations of viruses from well to well among those wells where viruses occur. As will be evident from the information presented, this variability encompasses both large scale differences between those wells considered to be less vulnerable and those considered to be more vulnerable, as well as differences from one location to another within each of these two categories of wells.

As noted previously, participants in the May 2005 statistics workshop were asked to consider how to model virus concentrations in virus-positive well water. Several options were considered both for stratifying the wells into different categories to reflect different ranges of expected concentrations and for fitting the concentration data to specific distributional forms to use in the baseline risk and risk reduction modeling. No specific recommendations were made.

Following the workshop, EPA decided to stratify wells into two categories according to overall vulnerability characteristics (more and less vulnerable wells). Unlike the hit rate analyses, which draw on data from 15 different studies, EPA relied upon only three key studies for viral concentration data. The data from the Lieberman et al. (2002) study are used to represent virus concentrations in more vulnerable wells and the combined data from the Abbaszadegan et al. (2003) study and the Pennsylvania study (Lindsey et al. (2002)) are used to represent concentrations from less vulnerable wells. The Lieberman et al. concentration data come from wells that were included in the study because they had a history of total coliform contamination or other evidence of vulnerability. As such, they are most like wells with TCR violations and therefore are assumed to be representative of this group of more vulnerable wells. The Abbaszadegan et al. 2003 study and the Pennsylvania study include wells selected for reasons other than a coliform occurrence history. As such, they are assumed to represent the less vulnerable wells group. The Pennsylvania wells are exclusively non-community wells and therefore the measured concentrations in these wells represent the group of less vulnerable non-community wells.

#### *Virus Concentration Data Used*

##### Concentrations for More Vulnerable Wells

EPA identified the Lieberman et al. 2002 study as providing the most complete set of virus concentration information for wells considered to be more vulnerable. These data are from cell culture assays for enteroviruses. As described in section 6.2, seven of the 30 wells in this study were found to have virus present by the cell culture method. A total of 20 positive values were observed. The concentrations of the positive values are presented in Exhibit 6.18 below (although data from GWUDI wells are shown below, the GWUDI well concentrations were not used in the primary risk and benefit analyses in the economic analysis).

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<sup>15</sup> Although hit rates were developed for both viruses and indicators, virus concentration modeling is necessary for the risk and benefits analysis, but *E. coli* concentration modeling is not. However, indicator hit rate information is needed to estimate risk reduction for the regulatory alternatives.

**Exhibit 6.18 Summary of Virus Concentrations Observed  
in the Lieberman et al. (2002) Study**

<b>Study Well Number</b>	<b>Concentration (PFU or MPN per 100 L)</b>
29	6.55
29	12.32
29	27.01
29	0.86
29	3.72
29	2.01
29	10.59
31	19.63
31	15.37
31	10.76
31	9.61
47	45.33
47	3.17
47	43.99
47	47.72
61	53.37
61	25.17
91	12.78
97	9.52
99	212.51

Note: Shaded rows indicate State-determined GWUDI wells.

### Concentrations for Less Vulnerable Wells

EPA identified the Abbaszadegan et al. (2003) and the Lindsey et al. (2002) studies as providing the most complete set of virus concentration information for wells considered to be less vulnerable.

In the Abbaszadegan et al. 2003 data, there were a total of 22 samples taken from 21 different wells with cell culture concentration data, as summarized in Exhibit 6.19 below.

**Exhibit 6.19 Summary of Virus Concentrations Observed  
in the Abbaszadegan et al. (2003) Study**

Study Well Number	Concentration (viruses per 100 L)
AZ-0001 / 3	1.89
AZ-0001 / 3	0.18
ID-0002	0.09
MO-0001	0.36
NH-1	0.19
IL-5	1.56
CA-1	0.45
PA-7	0.15
PA-21	0.17
NJ-13	0.17
CA-12	0.45
NJ-12	0.18
IL-10	0.18
IN-32	0.64
O-NY-15	0.18
O-WI-10	0.46
O-CA-22	0.92
O-CA-21	0.18
O-OH-6	0.19
OH-1	0.92
OH-3	0.15
IN-31	0.18

In the Lindsey et al. (2002) non-community well study, there were a total of 5 samples taken from 5 different wells with cell culture concentration data, as summarized in Exhibit 6.20 below.

**Exhibit 6.20 Summary of Virus Concentrations Observed  
in Lindsey et al. (2002)**

Study Well Number	Concentration (viruses per 100 L)
HU 425	0.21
JU 372	51.99
CE396	18.30
CH 5994	0.21
BR852	33.4

*Application of Virus Concentration Data for Baseline Risk and Risk Reduction Models*

As noted above, the participants in the May 2005 statistics workshop discussed alternative distributional forms to fit to the concentration data for use in the risk and risk reduction models. Following the workshop, EPA explored several options for fitting the data but determined that because of the limited number of data points and the considerable variability in the data even within the two vulnerability strata, that rather than fitting the data to a specific distributional form it was preferable to use the data directly and draw from them randomly, with replacement, in the simulation model.

Therefore, for the baseline risk and risk reduction simulation models described in the *Economic Analysis for the Final Ground Water Rule*, each well that is identified as having viruses present at some time has a concentration value drawn from one of the 7 non-GWUDI well values from the Lieberman et al. 2002 study if that well is in the more vulnerable stratum, and from one of the 27 values from the Abbaszadegan et al. 2003 and Pennsylvania studies if that well is in the less vulnerable stratum.

The concentration thus selected is assumed to be the average concentration in those samples or on those times when the virus is present. The use of these concentrations along with the  $P_{\text{sample}}$  value for the wells identified as having virus present is described in more detail in the economic analysis (USEPA 2006a).

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## Appendix A. Viral Pathogen Monitoring Methods Under Development

EPA believes that these methods, while promising, can still be considered to be “under development.” A wide range of new methods based on detection and manipulation of polynucleotides using polymerase chain reaction (PCR) are being developed. The methods are useful in research, but are too expensive and complex to use in the field at this time. The methods detect viable, non-viable, and viable, but non-culturable viruses, and even can be used to track pathogenic determinants (or genes) for pathogenic characteristics such as toxin production in *E. coli* (OECD 1999).

**Detection using direct PCR:** PCR is used to concentrate and amplify, to detectable levels, genetic material (DNA) from pathogens in water samples (Abbaszadegan 1999). Large volumes of water (100 to 1,500 liters (L)) are needed for a filter-adsorption and elution method. The result is a concentrate containing viruses, organic solids, and dissolved solids. PCR then uses high temperatures to denature the DNA molecules that are present (i.e., to unwind them into single strands). The temperature is then lowered to allow bonding of nucleotides (the smaller molecules that make up nucleic acids like DNA). Each of the four types of nucleotides bonds (via hydrogen bonding) to only one other type of nucleotide.

The enzyme DNA polymerase is added, along with free nucleotides and DNA primers, short sequences of nucleotides that are specific to the DNA of the virus or organism being assayed. These primers are building blocks that help the DNA polymerase get started. The primers bond (by hydrogen bonding) to the corresponding section on one of the single strands. DNA polymerase then helps attach free nucleotides to the ends of the primers to create new double strands.

The resulting DNA is heated and denatured, and more DNA polymerase is added, replicating the DNA again. This process is repeated multiple times until enough DNA is present to detect through gel electrophoresis or other methods. The detected gel profile is compared to a known DNA profile to confirm the correct virus has been detected. Thus, a positive PCR test indicates the presence of nucleic acid representative of the target organism. PCR is a relatively fast technique and is cheaper than virus cell culture assays. However, PCR detects DNA from both infectious or noninfectious viruses. Current PCR technology cannot yet determine whether a virus is viable or infectious.

**Detection using RT-PCR:** A second type of PCR is reverse transcription PCR (RT-PCR). Because many viruses have RNA as their genetic material rather than DNA, regular PCR does not work for detecting them. To detect these viruses, an intermediary step is needed between concentration and PCR itself. Reverse transcriptase, an enzyme that converts RNA to DNA, is added. PCR is then run on the resulting DNA. Both Abbaszadegan et al. and Reynolds et al. (2001) reported poor correlation between the results of cell culture and RT-PCR. Reynolds et al (2001) reported that one disadvantage of RT-PCR is that inhibitory compounds are naturally occurring, and reaction volumes are small, which can lead to false negatives.

**Detection using ICC/PCR:** The integrated cell culture PCR (ICC/PCR) method is a combination of cell culture and the direct- or RT-PCR methods described above. Rather than conducting PCR on the water sample itself, the laboratory adds the virus concentrate from the original sample to a cell culture. This allows the live viruses to multiply. The cell culture is then frozen and thawed to lyse the cells, and genetic material within the cells is used for PCR. Only viruses that replicated while the culture was growing should be detected.

ICC/PCR offers advantages in that it is both rapid and reliable, since the sequencing analysis adds confidence to the PCR positive results. This method only detects active viruses, is less time-consuming

1 (<48 hours as compared to 4 weeks), and is not as likely to produce false positive results, which are  
2 common with cell culture techniques.

3  
4 Since it is a combination of the previous two methods, ICC/PCR is significantly more expensive  
5 than cell culture alone. The costs of conducting PCR and ICC/PCR vary between laboratories based on  
6 the level of effort and time required to conduct the procedures. In the absence of robust sample controls,  
7 false negatives can occur. It may not be possible, therefore, to identify whether a sample is virus-negative  
8 due to a toxin in the sample (which can occur in both cell culture and nucleic acid formation methods)  
9 without rigorous sample controls. Sample controls can double the cost of the analyses. In addition, the  
10 following steps can be used to enhance purification of the sample. Purification minimizes interference  
11 from other chemicals that can affect both cell culture and PCR. The following steps each take time and  
12 effort and may or may not be used at various laboratories:

- 13  
14 • Molecular exclusion chromatography to purify virus concentrates and render them  
15 compatible with the diagnostic assay
- 16  
17 • Ethanol precipitation to concentrate viral RNA or DNA
- 18  
19 • PEG-precipitation to concentrate the viral particles
- 20  
21 • Centrifugal ultrafiltration to concentrate and purify viral particles
- 22

23 A significant disadvantage of ICC/PCR is that it is based on cell culture methods that may not always  
24 facilitate replication of the enteric viruses that were present in the original sample. Many pathogens are  
25 non-culturable in cell culture methods (more detail is provided in section 5.3).

26  
27 To further complicate the issue of identifying pathogens in environmental samples, researchers  
28 have demonstrated that some microorganisms lose culturability on appropriate media under certain  
29 conditions and, yet, still exhibit metabolic activity and, therefore, viability. In bacteria, this condition is  
30 called “viable but nonculturable (VBNC).” Alvarez (2000), studying poliovirus and MS-2 phage in  
31 ground water, found that a similar phenomenon exists among some viruses (although viruses do not  
32 metabolize), where viral reactivation can occur if environmental conditions change. The viruses are  
33 potentially infectious, but cannot be detected in traditional screening.

34  
35 **Costs:** Standard Method 9510 (cell culture) is estimated to have a commercial cost of  
36 \$600–\$1,000/sample concentrate based on a 50 to 100L sample. The cost does not include collection and  
37 shipping fees. The RT-PCR method has an estimated cost of \$250–\$500 for a sample concentrated based  
38 on a 50-1,500L sample and \$1,000 for a 100–3,000L sample. The ICC-PCR method includes a  
39 combination of the other two detection methods using a sample concentrate from a 50-1,500L sample at a  
40 cost estimated around \$1,500 and up (Battigelli 2001). The costs do not include collection costs and  
41 shipping fees.